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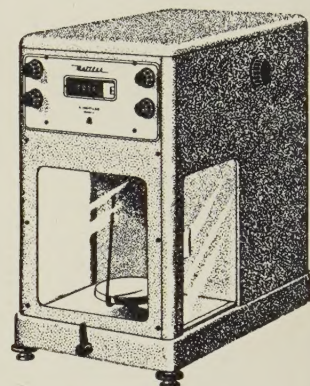
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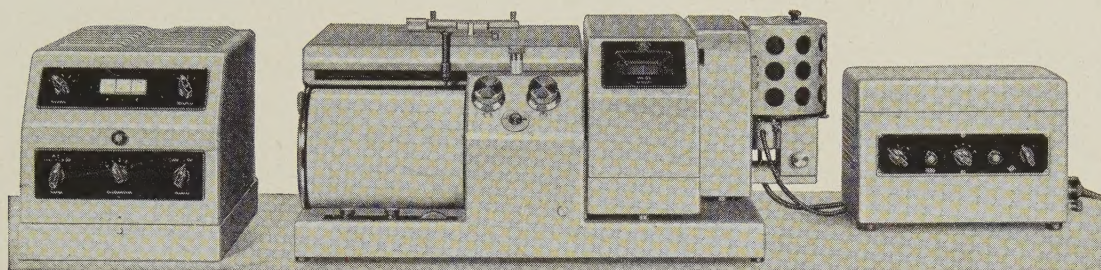
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The Phenocopy Concept: Illusion or Reality?

By W. LANDAUER*

The life history of scientific concepts, their birth, tumultuous adolescence and maturity, their ultimate senescence and oblivion, has received regrettably scant attention from those who fashion these concepts as their professional tools and who thereby become responsible for their use or misuse. Searching studies, such as FLECK¹ made of the syphilis concept, have done little to promote more general recognition that 'truth travels a variably curved path, and (that) scientists tend to travel in straight lines in the direction in which the thread pointed when they last had hold of it' (HARRISON²). The craving of the human mind for clear and sharp distinctions, the tendency to classify into either-or categories, continues to promote the use of spurious antitheses which by their apparent clarity hinder the progress of analytical thought and experimentation.

This danger is nowhere greater than in the field of biology. The physicist or chemist can in most instances safely operate with isolates, but the biologist must ever be on guard to remember the integrated nature of all living activities. The futile and naive controversies concerning the respective roles of form and function or about the relative importance of nature and nurture in the economy of organisms may seem amusing in retrospect; yet these pseudo-problems do, in fact, continue to plague clear thinking.

WOODGER's³ complaint that 'physiologists persist in confusing isolation in thought with isolation in nature' remains especially valid in regard to discussions concerning the influence of heredity and environment upon the realization of phenotypic traits. This is illustrated by much recent writing on the problem of 'phenocopies'. Naive adoption by some, uncritical rejection by others make it necessary to scrutinize the meaning and validity of the phenocopy concept.

GOLDSCHMIDT⁴ coined the term 'phenocopy' in 1935 as a designation for non-hereditary morphological

variants of *Drosophila* imagines, which he had obtained by treating definite larval stages with heat shocks, and which closely resembled known mutant traits. Simultaneous work by JOLLOS⁵ produced closely similar results and the same was true of 'X-ray morphoses' on which FRIESEN⁶ reported in 1936. All of these studies agreed in revealing in great detail an astonishing degree of similarity between the spectrum of mutants and the range of experimentally-induced non-hereditary variations. It became clear at once that the probability of this parallelism being due to chance was negligible. It remained, however, to be determined if the results were meaningful in the sense originally implied by the term phenocopy. The implication was well expressed by GOLDSCHMIDT⁷: 'Mutant and phenocopy, then, look alike because changed genetic action as well as action by a phenocopic agent is limited to definite tracks.'

HENKE, FINCK and MA⁸ were the first to suggest that it may be necessary to distinguish between 'true' and 'spurious' phenocopies. Their criterion for 'true' phenocopies was that the critical stage in development during which a particular modification can be produced experimentally should coincide with the time at which development of the homologous mutant deviates from the normal course of events. Later authors sought to base such distinctions on the condition that 'true' phenocopies should show a close and detailed parallelism with the corresponding mutants in the step by step alterations which lead to a particular variant. It seems to me, however, that neither of these two classifications carries much conviction. With reference to the time relationships, we know much too little about the real point of inception of mutant action to use it as a criterion for comparisons. But even when modifications clearly can be produced at a later stage than that at which the presumably corresponding mutant begins to deviate from the normal phenotype, it does not follow *eo ipso* that different developmental channels are involved. A particular mutant trait may, for instance, be brought about by insufficient production

Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Conn., USA.

¹ L. FLECK, *Entstehung und Entwicklung einer wissenschaftlichen Tatsache* (Benno Schwabe & Co., Basel 1935).

² G. R. HARRISON, *Atlantic Monthly*, June 1955.

³ J. H. WOODGER, *Biological Principles* (Kegan Paul, Trench, Trubner & Co., London 1929).

⁴ R. B. GOLDSCHMIDT, *Z. indukt. Abstamm. u. Vererblehre* 69, 38 (1935).

⁵ V. JOLLOS *Naturwissenschaften* 21, 831 (1933); *Genetica* 16, 476 (1934).

⁶ H. FRIESEN, *Arch. Entwicklungsmech.* 134, 147 (1936).

⁷ R. B. GOLDSCHMIDT, *Scientific American*, October 1949.

⁸ K. HENKE, E. v. FINCK, and S.-Y. MA, *Z. indukt. Abstamm. u. Vererblehre* 79, 267 (1941).

or complete lack of an essential compound at a specified developmental stage; a deficiency in the same substance, produced experimentally at a subsequent stage, may in genetically-normal sibs cause the regression or modification of parts that had already been formed and may subsequently, by identical physiological and morphological steps, be responsible for a phenotype closely mimicking the mutant trait. Such a situation would fulfill all the requirements that can reasonably be imposed on the phenocopy concept. Again, an agent acting from without might well achieve its mimicking end-result along routes that differ structurally from those followed by the mutant and yet do so by interference with the normal allele of the mutant. Contrariwise, it should be said that identity of the time of action or similarity of the developmental paths are by no means proof positive of an interference with the same genic activities. Mutant and modification might well produce homologous ends, and even along grossly similar routes, by an interference with quite dissimilar primary events⁹.

Many instances are on record in which the time sequence of events shows a satisfying correspondence between mutant and experimentally-produced modification. Cases of impressive similarity in the manner by which mutant and modified phenotype are reached have also been described (HADORN¹¹). Such examples may serve to strengthen our conviction that we are dealing with closely-related phenomena, but the decisive testimony must be furnished by evidence of a genetic nature. It is evidence of this kind which remains now to be examined.

Whenever an external agency impinges upon an organism, genetic forces of adjustment come into play. It is unlikely that such reactions occur at any time on the level of primary gene effects; in most instances it is, in fact, obvious that interpositions of this nature take place in reactions or events which are under indirect genic control, such as differential growth processes or enzyme-catalyzed steps of differentiation. Whatever is the precise mechanism by which experimentally-induced modifications occur, there is convincing evidence that the interference may be, and often is, in the same path by which recognized mutants produce homologous phenotypical results. This evidence is of the following kinds:

(1) Mutant genes and modifying agents which have similar phenotypic effects may, in conjunction, show *additive* activity. Such superposition has, for instance, been demonstrated by GOLDSCHMIDT⁴ in the case of scalloping of the wings of *Drosophila*.

(2) Animals which are heterozygous for a recessive mutant gene frequently give an enhanced response to modifying agents that produce phenotypic effects homologous to the trait shown by the mutant homozygotes. A clear demonstration of this kind was given by SANG and McDONALD¹² in their treatment with sodium metaborate of *Drosophila larvae* heterozygous for the recessive gene for eyeless. GOLDSCHMIDT and PITERICK¹³ and others have reported similar results. Recent (unpublished) experiments with fowl have shown: (a) that the treatment of chicken embryos with nicotine produces a shortening of the neck, due to abnormality of the cervical vertebrae, and that after high doses this defect is frequently accompanied by a shortening of the upper beak, hypoplasia of skeletal muscles (legs) and general edema; (b) that embryos heterozygous for a recessive lethal gene with very similar phenotype, the so-called 'crooked-neck dwarf' lethal, give a significantly higher response to treatment with nicotine than do related but non-heterozygous embryos; and (c) that embryos of a family of Brown Leghorn fowl in which abnormalities of the cervical spine are found on occasion (presumably as a mutant with very low penetrance) also react to nicotine with a higher incidence of the homologous malformation than do Brown Leghorn embryos of a family in which spontaneous occurrence of such defects had not been observed.

(3) The last-named of our observations on the results of treating chicken embryos with nicotine already belongs to the group of experiments on mutants with very low penetrance. GOLDSCHMIDT and PITERICK¹³ have reported several instances in which *Drosophila* mutants of this kind, e.g. *podoptera*, gave a high response to the experimental induction of comparable modifications. Another interesting example may be taken from the work of BERTSCHMANN¹⁴ on wing-scalloping in *Drosophila*. She worked with a stock which produced occasional individuals with slight scalloping, *provided* that the cultures were kept at low temperature. Starting from such individuals, she obtained by selection 100% penetrance and a high degree of expressivity of the scalloping phenotype, a trait which on analysis proved to be polygenic. When the unselected stock was treated with nitrogen mustard it produced a high incidence of wing scalloping – and, as in the controls, the reaction was temperature-dependent, low temperature favoring higher penetrance. BERTSCHMANN was clearly justified in concluding that a 'latente genetische Bereitschaft' for response to the external sources of variation (temperature and nitrogen mustard) was present. Another instructive

⁹ Doubts in regard to the distinction between true and spurious phenocopies have already been voiced by GOLDSCHMIDT¹⁰ and NACHTSHEIM²⁴.

¹⁰ R. B. GOLDSCHMIDT, *Theoretical Genetics* (Univ. California Press, Berkeley and Los Angeles 1955).

¹¹ E. HADORN, *Lethalfaktoren* (Georg Thieme, Stuttgart 1955).

¹² J. H. SANG and J. M. McDONALD, *J. Genetics* 52, 392 (1954).

¹³ R. B. GOLDSCHMIDT and L. K. PITERICK, *J. exp. Zool.* 135, 127 (1957).

¹⁴ M. BERTSCHMANN, *Z. indukt. Abstamm. u. Vererblehre* 87, 229 (1955).

example comes from our own work on rumplessness of fowl (LANDAUER¹⁵⁻¹⁷). This skeletal trait is known in three mimicking genetic forms, viz. as dominant (Rp-1) and recessive (rp-2) gene substitutions and as a more or less rare 'sporadic' variant which presumably has a polygenic background. The incidence of sporadic rumplessness varies in different stocks from a fraction of one to as much as 4 or 5%, but is typical for each stock and its residual genotype. The genetic nature of sporadic rumplessness was demonstrated by the effect which genotypes with dissimilar incidence have on penetrance and expressivity of the recessive mutant (rp-2). When the rumplessness-inducing effects of insulin were tested on various stocks, it was found that the incidence was directly related to, i.e. increasing with, the frequency of occurrence of sporadic rumplessness. We are, therefore, again dealing with a situation in which the response to external modifying agencies depends intimately on the extent to which the genotype favors homologous variation.

(4) Further evidence for the relatedness of genic activity and of response to modifying agents with homologous phenotypic effects comes from observations on the role which genetic modifiers may play in both situations. It has been shown repeatedly in our work with chickens that the accumulation of modifying genes which reduce penetrance and expressivity of a mutant lowers in a similar manner the response to external forces which are accountable for homologous modifications of development. Even after the phenotypic expression of a gene in *Drosophila* had become completely suppressed by genetic modifiers, its presence in the genotype played an important role in determining the measure of reactivity to an external force favoring a corresponding developmental modification (GOLDSCHMIDT and PITERNICK¹³). Again, it has been shown that sexual differences in the expressivity of a particular trait are in a similar manner reflected in sexual dissimilarities of response to forces from without (SANG and McDONALD¹²).

(5) An interesting example of modifying influences exerted by the maternal environment can be found in the beautiful studies of McLAREN and MICHIE¹⁸ on vertebral variation in mice. They established the following facts. Two inbred strains of mice differ in the mean number of lumbar vertebrae, C3H/Bi having mainly five and C57BL/How mainly six of these vertebrae. Reciprocal hybrids resemble in both sexes the maternal strain; the trait is, therefore, not sex-linked. Transfer of F₁ zygotes from reciprocal crosses to the uteri of females of either one or the other of the two strains produced young with vertebral counts

typical for the host-mother inbred stock. In the last analysis the two strains clearly differ in genetic factors which are, however remotely, responsible for dissimilarities of uterine functions during pregnancy, thereby determining the number of lumbar vertebrae in their progeny. It is equally evident that after egg transfer the uterine environment modifies development of reciprocal F₁-hybrids in such a way as to produce an exact duplication of the features which are typical for the host strain.

Available evidence demonstrates, therefore, that (1) genic and external factors may produce additive effects; (2) organisms which are heterozygous for a recessive mutant gene frequently give heightened response to modifying factors with homologous developmental effects; (3) mutants with low penetrance, similar to single doses of recessive genes, often promote reactivity to external modifying action; (4) modifying genes may produce closely similar results in combination with mutants or external sources of homologous variation; (5) the uterine environment of a host-mother may serve as a source of homologous variation. In all situations in which intimate relations of this kind can be observed between genic activity and the manner in which external agencies interpose their effects on development, we can confidently conclude that the concept of phenocopy, as indicating the use in both instances of closely similar development tracks, *if probably only part of the way*, is born out by experimental evidence. The tracks must, in any event, be sufficiently related to accommodate in similar manner the directing motions of the variety of genic modifying forces to which reference has been made.

It is obvious, of course, that one should avoid using the term phenocopy indiscriminately. It is true, as GOLDSCHMIDT has pointed out, that in many instances of experimentally-produced variants without hereditary counterpart, homologous mutants may be found subsequently. Remarkable examples of this kind have been reported in the field of vitamin deficiencies in man (SYNDER¹⁹). The question remains if, in addition to cases in which a verdict of 'not proven' is in order, there are situations in which the concept 'phenocopy' is inapplicable. I believe this to be true whenever the events by which a developmental variant is produced clearly are of a nature that has nothing in common with the manner in which any gene-directed process might lead to similar endresults. A case in point are, I believe, the malformations produced by German measles and other viruses. FRANCESCHETTI, BAMATTER, and BOURQUIN²⁰ and TÖNDURY²¹ have referred to the rubeola-induced defects of human fetuses as phenocopies. But since it has been shown by HAMBURGER and

¹⁵ W. LANDAUER, Growth Symposium 12, 171 (1948).

¹⁶ W. LANDAUER, Amer. Naturalist 89, 35 (1955).

¹⁷ W. LANDAUER, Amer. Naturalist 91, 79 (1957).

¹⁸ A. McLAREN and D. MICHIE, J. Embryol. exp. Morphol. 6, 645 (1958).

¹⁹ L. H. SNYDER, Science 129, 7 (1959).

²⁰ A. FRANCESCHETTI, F. BAMATTER and J. B. BOURQUIN, Helv. paediat. Acta 2, 339 (1947).

²¹ G. TÖNDURY, Arch. Julius-Klaus-Stiftung 27, 170 (1952).

HABEL²², TÖNDURY²³ and others that in these cases damage to the embryo is brought about by viral invasion and destruction of certain embryonic organs and tissues, it is difficult to conceive of any common developmental denominator between these events and comparable end-effects of mutant conditions, even if the latter should come about by cellular destruction and resorption. The same applies *a fortiori* to the results of destruction by surgery or X-rays of circumscribed embryonic areas. A more complex situation exists in the case of sex reversal of female fowl, to which NACHTSHEIM²⁴ has referred as phenocopies of males. As after viral infection, the primary event is the destruction of an organ (the ovary) by a process (tubercular infection or malignancy) which has no equivalent in genetic conditions, but the physiological events which are initiated thereby are those determined by the residual genetic constitution.

The question whether the reactions of organisms to external insults are, however indirectly, governed by their genetic constitution was not an issue in our discussion. That is an evident fact, based on the inseparability of action and reaction as a consequence of evolutionary integration. The question at issue was whether it is justified to use the term phenocopy with the implication that external sources of modification and genes with similar phenotypic expression reach their goals by developmental routes which are, at least

in part, identical. It has been shown that a considerable amount of genetic evidence is available in support of this conclusion. It should be recognized that in any specific situation the phenocopy concept must remain shadowy until its relevancy is clearly established.

There is no proof that external sources of developmental variation ever produce their results by interference with primary gene functions. It would be a mistake to interpret the phenocopy concept in this manner, and any expectations based on such implications will surely prove illusory. Again, mere similarity between the end-results of mutant action and of those produced by external agencies is not enough to make the phenocopy concept meaningful to geneticists. But if we accept the concept in its restricted meaning, viz. as designating identical or closely similar phenotypes reached by paths which, part of the way, are shared with those of the homologous mutants, then existing evidence validates its reality, and in this sense work on phenocopies may become a valuable tool in our search for some of the developmental mechanisms by which mutant genes produce their phenotypic effects.

Zusammenfassung

Verfasser bespricht den Begriff Phänokopie und dessen Grenzen. Er kommt zum Schluss, dieser Begriff sei nützlich, sofern er sich auf experimentell produzierte Phänokopien bezieht, die mit den Erbphänotypen identisch oder ihnen sehr ähnlich sind und deren Entwicklungsgang zum mindesten teilweise mit demjenigen homologer Mutanten übereinstimmt.

²² V. HAMBURGER and K. HABEL, Proc. Soc. exp. Biol. Med. 66, 608 (1947).

²³ G. TÖNDURY, Geburtsh. u. Frauenheilk. 12, 865 (1952); Helv. paedit. Acta 7, 105 (1952).

²⁴ H. NACHTSHEIM, Exper. 13, 57 (1957).

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

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Über die Orientierung bei der Kondensation von Benzil mit monosubstituierten Guanidinen¹

Bekanntlich lässt sich die alte Biltzsche Synthese³ von 5,5-Diaryl-hydantoinen und -2-thiohydantoinen aus Benzilen und Harnstoff bzw. Thioharnstoff in Gegenwart von Alkalien auch auf die Synthese von 5,5-Diaryl-glykocynamidinen (I, $R=R'=H$) anwenden, wenn Harnstoff

bzw. Thioharnstoff durch Guanidin ersetzt wird⁴. Der einzige bisher ausgeführte Versuch zur Übertragung dieser Kondensation auf *substituierte* Guanidine, wie 1,3-Diphenyl-guanidin⁵, blieb erfolglos; ebenso der Versuch der analogen Kondensation des N-Butylguanidins mit Glyoxal⁶.

Wir kondensierten Benzil mit zwei monosubstituierten Guanidinen, dem N-Benzyl- sowie dem N-(β -Morpholino-äthyl)-guanidin durch Kochen der alkoholischen Lösung in Gegenwart von Kaliumhydroxyd. Bei Verwendung von

¹ 5. Mitteilung über Hydantoine, Thiohydantoine und Glykocynamidine².

² 4. Mitteilung siehe K. LEMPert und J. BREUER Chem. Ber. 92, 1710 (1959).

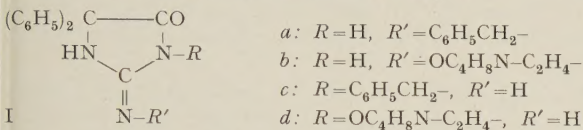
³ Zusammenfassende Darstellung: E. WARE, Chem. Rev. 46, 414 (1950).

⁴ CH. HOFFMANN, Bull. Soc. Chim. France 1950, 659. - C. V. DELIVALA and S. RAJAGOPALAN, Proc. Indian Acad. Sci. (A) 31, 107 (1950).

⁵ R. G. NEVILLE, J. org. Chem. 23, 1588 (1958).

⁶ I. S. BENGELSDORF, J. chem. Soc. 75, 3138 (1953).

50 Mol% KOH erhielten wir in einer Ausbeute von 60 bis 70% 2-Benzylimino-4,4-diphenyl-imidazolidin-5-on (Ia, weisse Nadeln, Smp.⁷ und Misch-Smp. mit authentischem Präparat⁸: 240–241°C), bzw. 2-β-Morpholinoäthylimino-4,4-diphenyl-imidazolidin-5-on (Ib, weisse Nadeln, Smp. und Misch-Smp. mit authentischem Präparat⁹: 194 bis 195°C). In Stellung 3 substituierte Isomeren (Ic bzw. d) konnten im Reaktionsprodukt nicht aufgefunden werden.



Wurde jedoch die Menge des eingesetzten Kaliumhydroxids auf 10 Mol% verringert, so erhielten wir im Falle des Benzylguanidins als Reaktionsprodukt ein Gemisch, aus welchem durch Chromatographie an Brockmannschem Aluminiumoxyd neben 20% d. Th. Ia 45% d. Th. des isomeren 3-Benzyl-5,5-diphenyl-glykocyamidins (Ic, weisse Plättchen, Smp. und Misch-Smp. mit authentischem Präparat⁸: 164–166°C) isoliert werden konnten. Im Falle des N-β-Morpholinoäthyl-guanidins wurde durch einfache Kristallisation des Reaktionsproduktes das 3-β-Morpholinoäthyl-5,5-diphenyl-glykocyamidin (Id, weisse Prismen, Smp. und Misch-Smp. mit authentischem Präparat¹⁰: 198–199°C) in einer Ausbeute von 60% erhalten; das isomere Ib liess sich im Reaktionsprodukt nicht auffinden. In *Abwesenheit* von Kaliumhydroxid konnten 59% Ic, hingegen kein Ia isoliert werden. Für die Kondensation von Benzil mit Guanidinen zu Glykocyamidinen ist also die Verwendung eines besonderen alkalischen Kondensationsmittels nicht immer notwendig.

Da, wie aus besonders angestellten Versuchen hervorgeht, Ic beim Kochen seiner alkoholischen Lösung in Gegenwart von 1/2 Mol Kaliumhydroxid teilweise zu Ia umgelagert wird, kann der Einfluss des Kaliumhydroxids auf die Orientation bei der Kondensation vielleicht durch die Annahme einer *nachträglichen* Umlagerung der primär stets gebildeten 3-substituierten 5,5-Diphenyl-glykocyamidine durch das Kaliumhydroxid in die entsprechenden 2-(subst. Imino)-4,4-diphenyl-imidazolidin-5-one erklärt werden.

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Summary

Condensation of benzil with two monosubstituted guanidines in the presence of 50 mol% of potassium hydroxide leads to the formation of 2-(subst. imino)-4,4-diphenyl-imidazolidin-5-ones (Ia, b); by reduction of the potassium hydroxide used the formation of these products is more or less driven back in favour of the formation of 3-substituted 5,5-diphenyl-glykocyamidines (Ic, d). A possible explanation for these orientation phenomena is given.

⁷ Alle Schmelzpunkte unkorrigiert.
⁸ K. LEMPERT, J. BREUER und M. LEMPERT-SRÉTER, Chem. Ber. 92, 235 (1959).
⁹ Dargestellt durch Aminolyse des S-Methylderivates des 5,5-Diphenyl-2-thiohydantoin's mittels β-Morpholinoäthylamin (eigener unveröffentlichter Versuch).
¹⁰ K. LEMPERT, J. BREUER, M. LEMPERT-SRÉTER, I. PATAKY und K. PFEIFER, Magyar Kém. Folyóirat (Ung. Z. Chem.) 65, 110 (1959).

Effect of Mg⁺⁺
on the Reactivity of Ribonucleic Acid¹

On the basis of spectrophotometric, optical rotation, and sedimentation data HASCHEMEYER *et al.*² have concluded that tobacco mosaic virus nucleic acid (TMV-RNA) may exist in two forms showing a random coil configuration in water and a more structured configuration in the presence of salts. One of the most characteristic features of the latter is its markedly lower ultraviolet absorption (hypochromicity). Salt dependent changes in configuration accompanied by a decrease in ultraviolet absorption have also been found in synthetic polyribonucleotides^{3,4}, and are believed to be brought about by purine pyrimidine hydrogen bonds similar to those in DNA^{5,6}. If a similar bonding occurred in ribonucleic acid the change in configuration could well be reflected in the reactivity of the bases which might become unreactive in the hydrogen bonded state. Furthermore, the quantitative determination of the extent to which the bases become unreactive would seem important for a closer understanding of the changes which take place in the configuration of RNA.

Table I
Formaldehyde binding and hypochromic effect at various ratios of MgCl₂ to RNA-phosphorus

Mg ⁺⁺ RNA-P	Formaldehyde bound RNA-Amino groups	Hypochromic Effect* Δε 260 mμ
0 (no Mg)	0.95	—
0.0003	0.99	—
0.03	0.97	— 3.5%
0.3	0.50	— 13.5%
3	0.36	— 14.5%

* The hypochromic effect was determined by adding 0.1 ml of various MgCl₂ solutions to 0.9 ml of a TMV-RNA solution.

0.5 mg of TMV-RNA was incubated with 0.1% formaldehyde C¹⁴ (containing 850 c.p.m./μM) at room temperature (23°C) for 48 h. Total volume 0.5 ml. The samples were then cooled and the nucleic acid precipitated with 2 vol. of cold alcohol and a drop of 3 M ammonium acetate pH 5.0. The sediment was taken up in 0.3 ml of icecold water and reprecipitated 5 times to remove all unbound formaldehyde. Care was taken to keep the temperature always at or below 0°C. Of the final solution of 0.3 ml in water 0.2 ml were placed in a counting dish and dried *in vacuo*, and 0.005 ml were diluted to 1 ml and used for the determination of the absorbance at 260 mμ.

Formaldehyde was shown in an earlier report⁷ to react with the amino groups of the bases of RNA. The extent to which the reaction occurs in the presence and absence of Mg⁺⁺ was therefore studied (Table I), and it became evident that less formaldehyde was bound by the structured than by the random coil form. HASCHEMEYER *et al.* have found that the Mg⁺⁺ concentration at which the change to the more structured form of RNA takes place

¹ Aided by a grant from The National Foundation.
² R. HASCHEMEYER, B. SINGER, and H. FRAENKEL-CONRAT, Proc. nat. Acad. Sci., Wash. in press.
³ R. C. WARNER, J. biol. Chem. 229, 711 (1957).
⁴ G. FELSENFELD, D. R. DAVIES, and A. RICH, J. Amer. chem. Soc. 79, 2023 (1957).
⁵ A. RICH and D. R. DAVIES, J. Amer. chem. Soc. 78, 3548 (1956).
⁶ J. D. WATSON and F. H. C. CRICK, Cold Spring Harbor Symposia quant. Biol. XVIII (1953).
⁷ M. STAEHELIN, Biochem. biophys. Acta 29, 410 (1958).

is a function of the RNA concentration, the reaction approaching completion when 0.5–1 molecule of Mg^{++} is present per molecule of RNA phosphorus. In Table I the results are therefore presented on the basis of a Mg^{++} /RNA-P ratio and show a parallelism between the formaldehyde reaction and the hypochromic effect. From a quantitative point of view it may be interesting to note that in the absence of salts the same number of formaldehyde molecules are bound as there are amino groups present and that this amount is reduced by about 65% in the presence of higher Mg^{++} concentrations. Similar values have been obtained with TMV-RNA in 10^{-2} to 10^{-1} M potassium chloride as well as with TMV-RNA prepared by means of metal containing phenol².

This strongly suggests that the amino groups of the purine and pyrimidine bases are involved in the formation of this more structured form of RNA. Mg^{++} has been shown to favour the formation of double and triple stranded helices through hydrogen bonds between base-pairing polyribonucleotides^{3,4}, probably through its effect on the negative charges of the phosphate groups. The amino groups lose their reactivity under these conditions⁷. From an analogy with these models it may be inferred that a base pairing of the purine and pyrimidine bases takes place in RNA also. If this were the case the results would indicate that about 80% of all the theoretically possible hydrogen bonds have been formed, since purines are present in about 18% excess over the pyrimidines in TMV-RNA⁸. Not all the bases, therefore, could become involved in hydrogen bonding. The unreactivity of the bases does not necessarily permit the conclusion, however, that hydrogen bonds have been formed since other ways are also conceivable by which the amino groups might be blocked. ZUBAY⁹ has recently postulated that in denatured DNA Mg^{++} might chelate with the adenine and guanine bases. But it seems unlikely that this would be the way by which Mg^{++} changes the configuration of RNA, since similar changes in configuration have also been observed by higher concentrations of monovalent ions and since under these conditions the hypochromic effect is not reversed by versene¹⁰.

Table II

Hypochromic effect in the presence of various formaldehyde and Mg^{++} concentrations (ϵ 260 m μ)

Formaldehyde concentration	H ₂ O	$3 \times 10^{-6} MgCl_2$	$10^{-5} MgCl_2$	$10^{-4} MgCl_2$
—	0.940	0.930	0.840	0.820
0.1%	0.940	0.930	0.840	0.820
0.2%	0.950	0.945	0.860	0.840
0.5%	0.980	0.970	0.900	0.875
1.0%	0.990	0.985	0.970	0.950

Yeast RNA¹¹ was incubated for 48 h at room temperature with the concentrations of formaldehyde and $MgCl_2$ indicated and the absorption at 260 m μ determined. Glass distilled water was used throughout the experiment

Interestingly in our experiments we have found no changes in the ultraviolet spectrum of ribonucleic acids upon treatment with formaldehyde in contrast to the

earlier report by FRAENKEL-CONRAT¹². The difference can be accounted for by the different concentrations of formaldehyde which were used. The effect of various concentrations of formaldehyde upon the optical properties of nucleic acid is shown in Table II. Formaldehyde in concentrations of 0.1% which earlier experiments⁷ have shown to suffice for complete reaction of all amino groups of the nucleic acid do not cause any increase in its ultraviolet absorption. An increase occurs only with higher concentrations and is especially evident in the presence of Mg^{++} .

It seems likely that at low concentrations formaldehyde reacts by forming a monomethylol derivative ($-NH-CH_2OH$) whereas at higher concentrations a dimethylol derivative ($-N=(CH_2OH)_2$) is formed which would be responsible for the spectroscopic changes. The larger increase in the presence of Mg^{++} could be an indication that formaldehyde at higher concentrations causes a reversal of the salt dependent change in configuration of RNA¹³. The absence of any spectroscopic changes at low concentrations indicates that under these conditions formaldehyde does not alter in any way the configuration of the RNA molecule. The stoichiometric relationship between the formaldehyde bound and the amino groups present as well as the evidence that under the conditions of the reaction (0.1%) only the monomethylol derivative is formed lend further support to the conclusion that in a salt free medium all amino groups are free and reactive and the reduction in formaldehyde binding by 65% upon the addition of Mg^{++} is a true expression of the number of amino groups which through hydrogen bonding or by some other means have become unreactive.

The author wishes to thank Dr. W. M. STANLEY and Dr. H. FRAENKEL-CONRAT for their interest and stimulating criticism.

M. STAEHELIN*

Virus Laboratory, University of California, Berkeley, September 3, 1959.

Zusammenfassung

Die Nukleinsäure des Tabakmosaikvirus kann in zwei Formen vorliegen. In salzfreiem Milieu sind sämtliche Aminogruppen frei und reagieren mit Formaldehyd, während in Gegenwart von Mg^{++} nur etwa 35% reaktionsfähig bleiben. Diese Veränderung, die parallel mit dem hypochromen Effekt einhergeht, deutet darauf hin, dass unter dem Einfluss von Mg^{++} die Basen gegenseitige Beziehungen eingehen und dadurch dem Molekül eine geordnete räumliche Konfiguration geben.

¹² S. ZAMENHOF, G. GRIBOFF, and N. MARULLO, Biochem. biophys. Acta 13, 459 (1954).

¹³ This might explain why formaldehyde, although at 0.1% it does not react with DNA, leads to inactivation of the transforming principle of bacteria at 3%¹¹.

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Alkaloids of Apocynaceae IV¹. Dregamine, a New Alkaloid from *Voacanga dregei* E. M.

In continuation of our studies of alkaloids from different species of the family *Apocynaceae*, we have examined a

⁸ K. K. REDDI, Biochem. biophys. Acta 23, 208 (1957).

⁹ G. ZUBAY, Biochem. biophys. Acta 32, 233 (1959).

¹⁰ H. FRAENKEL-CONRAT, Personal communication.

¹¹ A. M. CRESTFIELD, K. C. SMITH, and F. W. ALLEN, J. biol. Chem. 216, 185 (1954).

¹ Alkaloids of Apocynaceae III, M. GORMAN, N. NEUSS, J. A. DEYRUP, and N. J. CONE, J. Amer. chem. Soc. (1959), in press.

new species of the genus *Voacanga*, *Voacanga dregei* E.M. We wish to report the isolation of a new alkaloid, dregamine, from the bark of this tree².

Dregamine is a representative of 2-acyl indole alkaloids³; it was isolated⁴ by chromatography of alkaloids obtained by benzene extraction of the bark of the trunk put at our disposal through the cooperation of Mr. J. L. SIEVEY (Pietermaritzburg, Natal, S. Africa), who also established the botanical authenticity of the plant material with herbarium specimen (fruit and leaves). Elution with benzene-chloroform mixture (3:1) gave the crude base which crystallized from methanol in long prisms; m. p. 106–109°C, resolidified, then m. p. 186–205°C (dec.); $[\alpha]_D^{25} = -93.1^\circ\text{C}$ (CHCl_3 , $C = 1$).

Calculated for $\text{C}_{21}\text{H}_{28}\text{O}_3\text{N}_2$: C 70.76; H 7.92; N 7.86. Found: C 70.57; H 7.47; N 7.42. The hydrochloride was prepared in the conventional manner and recrystallized from methanol-ether, m. p. 249–250°C (dec.). Calculated for $\text{C}_{21}\text{H}_{28}\text{O}_3\text{N}_2 \cdot \text{HCl}$: C 64.19; H 7.74; N 7.13; Cl 9.02; OCH_3 (1) 7.90; (N)- CH_3 (1) 3.83. Found: C 64.49; H 7.62; N 7.00; Cl 8.98; OCH_3 8.12; (N)- CH_3 3.84.

The ultraviolet spectrum of dregamine is characterized by the following bands: $\lambda_{\text{max}}^{\text{EtOH}}$ 239 m μ , $a_M = 15,200$; 316 m μ , $a_M = 18,600$ and very similar to that of 1-keto-1,2,3,4-tetrahydrocarbazole³. In addition to the 2-acyl indole moiety (major band at 6.05 μ)¹, the infrared spectrum indicated the presence of a carbomethoxyl moiety with absorption at 5.78 μ and 8.03 μ ⁵.

Other botanically related genera are currently under investigation in these laboratories.

Acknowledgment. The authors are grateful to Dr. H. E. BOAZ for the infrared data, Mr. L. G. HOWARD for the ultraviolet data, and Messrs. W. L. BROWN, R. HUGHES, H. L. HUNTER, and G. M. MACIAK for microanalyses.

N. NEUSS and NANCY J. CONE

Lilly Research Laboratories, Indianapolis (Indiana), July 6, 1959.

Zusammenfassung

Dregamine, ein neues Alkaloid aus der Apocynaceae *Voacanga dregei* E. M. wurde isoliert und charakterisiert. Diese Verbindung stellt einen neuen Vertreter der Klasse der 2-Acyl-indole dar.

² After the completion of this work, SCHULER, VERBEEK, and WARREN, J. chem. Soc. 1958, 4776, have reported the isolation of the known alkaloids vobtusine and voacangine from the bark of *Voacanga dregei* collected in the South Coast, Natal, S. Africa. We were unable to find these alkaloids in our plant material.

³ M. GORMAN, N. NEUSS, and N. J. CONE, Amer. chem. Soc. nat. Meeting, San Francisco, Calif., April 1958. The spectral data reported for voacafrine and voacaficine [K. V. RAO, J. org. Chem. 23, 1455 (1958)] are also indicative of the presence of a 2-acyl indole moiety in these two alkaloids from *Voacanga africana*.

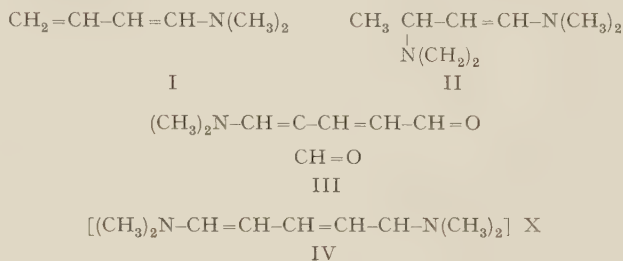
⁴ Small amounts of this alkaloid were also isolated from *Ervatamia coronaria*³.

⁵ Subsequent to the preparation of this manuscript, RENNER has described the isolation of vobasine and voacryptine from *V. africana*. The former alkaloid appears to be another representative of 2-acyl indoles³ [U. RENNER, Exper. 15, 185 (1959)].

A Simple Synthesis of Nicotinic Aldehyde

Practically all known routes leading to nicotinic aldehyde involve the reduction of various nicotinic acid derivatives. We have now found a simple and advantageous method for the synthesis of this valuable compound based on crotonaldehyde.

Recently we have shown^{1,2} that the Vilsmeier-Haack reaction may be extended to the aliphatic series, in particular to carbonyl compounds. By this reaction, we have prepared a large number of β -dicarbonyl derivatives, such as the β -dialdehydes, β -chlorovinylaldehydes and some novel types of polyformyl derivatives. Most of these compounds had previously been difficult of access.



We have now applied the reagent prepared from dimethylformamide and phosgene to compounds I and II, which are readily available from crotonaldehyde in a single step^{3,4}. This reaction led to the trialdehyde derivative III in about 60% yield. This derivative is even more simply obtained by formylation of the well known quaternary salt IV which is also believed to be an intermediate in the formylation of I and II. The trialdehyde derivative III very readily passes into nicotinic aldehyde in excellent yield, e.g. merely on heating with aqueous NH_4Cl .

Nicotinaldehyde is thus available from crotonaldehyde in a simple three-step process in about 40% overall yield.

Z. ARNOLD

Department of Organic Synthesis, Institute of Chemistry, Czechoslovak Academy of Science, Prague, July 17, 1959.

Zusammenfassung

Nikotinaldehyd wurde durch ein dreistufiges Verfahren aus Krotonaldehyd in einer Gesamtausbeute von 40% erhalten.

¹ Z. ARNOLD and F. ŠORM, Coll. Czechoslov. chem. Comm. 23, 452 (1958). – Z. ARNOLD and J. ŽEMLIČKA, Coll. Czechoslov. chem. Comm. 24, 786, 2378, 2385 (1959); 24, in press.

² Z. ARNOLD and J. ŽEMLIČKA, Proc. chem. Soc. 1958, 227.

³ C. MANNICH, K. HANDKE, and K. ROTH, Chem. Ber. 69, 2112 (1936).

⁴ W. LANGENBECK and L. WESCHKY, DRP 715544, Chem. Zbl. 1942, 2821.

Demonstration of Poliomyelitis Virus in Homogenates and Ureadesoxycholate Lysates of Cells Exhibiting or Lacking Cytopathogenicity

A method for dissolution of tissue cultures (TC) with Ureadesoxycholate (UDC) was described previously¹. Ap-

¹ E. Kovács, Naturwissenschaften 45, 339 (1958); Arch. Biochem. Biophys. 76, 546 (1958).

Table. Infectivity of TC Homogenates or lysates

Total of Tubecultures pooled for assays	Type of fraction assayed	No. of cultures inoculated: time and grade of CPE	No. and state of normal controls	Remarks
1. 120 CPE-negative HeLa and 30 human amnion cell-cultures in complete medium; 14 days inoculation with Mahoney Str. 10^{-2} to 10^{-9} dilution	Pooled, centrifuged supernatants after thermal shock (storage 4°C , reincubation to 37°C)	2 HeLa Flask-cultures, 5 ml undiluted pool 30 min adsorption and 10 ml complete medium. CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
2. 120 CPE-negative HeLa and 30 human amnion cell-cultures in complete medium; 14 days inoculation with Mahoney Str. 10^{-2} to 10^{-9} dilution	Pool of homogenates (cells stored at -20°C , without culture-fluid)	2 HeLa Flask-cultures, 5 ml undiluted pool 30 min adsorption and 10 ml complete medium. CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
3. 49 CPE-negative HeLa and 10 human amnion cell-cultures, as above	Pool of 10% diluted Urea-lysate of cells (stored at -20°C)	5 ml Lysate in 10^{-3} dilution: procedure and results as above	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
4. About 120 CPE-negative HeLa cell-cultures, as above	'Total lysates': frozen cells dissolved in Urea-reagent and diluted to 10% with own medium + H_2O	Assayed after 7 weeks storage at 4°C , Delayed CPE 4-plus, suddenly on 7th day	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
5. 60 CPE-negative HeLa cell-cultures, as above	'Total lysates' as above	Assayed immediately; CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive
6. 30 CPE-positive HeLa cell-cultures as above	Supernatant separated; cell residue lysed and diluted till 10% Urea-concentration	Assayed immediately; CPE 4-plus in 2 to 3 days	Two HeLa Flasks 5 ml normal TC-fluid and 10 ml compl. medium; cells intact	Second passage: positive

plication of the same techniques for demonstration of infectious virus in cell cultures *unsuccessfully* inoculated with poliomyelitis virus is now reported. Characteristic enzyme changes were observed in similar TC suggesting the viral cause of biochemical alterations². The following experiments demonstrate that superficial or intracellular association of highly cytopathogenic virus (Type I, Mahoney str.) may occur *without massive cell destruction and cytological changes* visible on simple microscopic inspection. About 120 tubes of individual subcultures of HeLa and primary cultures of human amnion cells exposed to virus dilutions from 10^{-2} to 10^{-9} were collected which *did not show any signs of cytopathogenic effect* (CPE), even after 10 to 14 days incubation at 37°C . These TC's were allowed to stand 2 days at 4°C , then 2 days at 37°C . The supernatants were pooled, centrifuged, and assayed separately. The unwashed cells were homogenized by a loosely fitting motor-driven pestle of an all-glass micro-grinder (Potter-Elvehjem type) in about 1 min. runs. The

pooled homogenates were assayed for infectivity; two TC's, each with ab. 3×10^6 HeLa cells grown with 15 ml complete medium³ were inoculated with 5 ml undiluted 'brei'. Two other cultures received 5 ml of the pooled supernatants to test the presence of virus in the fluid-phase. Usually 30 min adsorption of the inoculum was allowed at 37°C , then 10 ml fresh medium was added and the TC reincubated at 37°C . Inspection after 18 h revealed definite CPE leading to total destruction of the TC's within 2-3 days. It was concluded that both the pools of *supernatants* and *homogenates* contained highly infectious virus material.

This suggestion was proved by chemical means. 60 CPE-negative TC's of HeLa and primary human amnion cells were frozen without nutrient-fluid at -20°C , then dissolved by UDC-reagent² to demonstrate cell-associated virus. 2 ml UDC were added to five tubes at room temperature. The cultures lysed promptly and were poured over into other tubes. This procedure was repeated several times back and forth, finally all lysates were pooled, the tubes

² E. Kovács, Z. Vitamin-, Hormon-Fermentforsch. 4 (1959).

³ J. L. MELNICK, Ann. N. Y. Acad. Sci. 61, 754 (1955).

washed with H_2O , the lysates diluted 10-fold (10% final urea conc.) with the rinsing-water² as soon as possible and stored at 4°C. The whole procedure involved about 60 min. For inoculation, a 10^{-3} dilution¹ with bovine amnion fluid was made and assayed as described. Control flasks received similarly diluted UDC. The TC, exposed to lysates were destroyed within two days, whereas controls treated with urea-desoxycholate alone remained intact, permitting growth and subcultivation of the cells. It seemed that the lysates were carrying (among others) *biologically active virus material* set free by UDC and preserved by the timely dilution². This assumption was substantiated by the following experiments. *The cell residues of positively infected and destroyed (CPE-positive) TC* were dissolved in UDC as usual and their preserved infectivity demonstrated. HeLa cells inoculated with this lysate exhibited visible CPE after 16 h and the whole culture was destroyed within two days. Finally if 10% Urea-lysates were added to the TC and after 5 min diluted 3 times with amnion fluid, immediate destruction of the cells by the UDC occurred. This was proved with the large 'total' pool illustrated in the Table (group 4) where medium and cells were lysed together. The tenfold diluted lysates, however, do not retain intactly their infectivity during 7 weeks storage at 4°C; if tested in 10^{-3} dilution, CPE was not apparent only after 6 days, when overnight total destruction of the inoculated TC occurred. Second passages of the cultures destroyed were positive, confirming the presence and successful transmission of infective material with homogenates and lysates (Table). Concentration, purification, and isolation of the active principle is under study⁴.

We might summarize that the above findings were repeatedly observed and may prove the following assumptions: (1) The CPE-negativity of unsuccessfully infected TC may depend on a peculiar behaviour of the cells against polio virus (2). The infective particle seems to be firmly associated to certain cells without apparent cytopathogenic effect, and can be liberated by *physical* (thermal-shock, grinding) and/or *chemical* means (3). UDC-treatment *did not destroy the biological activity* of the virus examined (4). The UDC reagent seems to be the simplest *chemical* tool⁵ for the liberation of infectious material from true or latent poliomyelitis infection *in vitro*, although the nature of this procedure has to be clarified.

The technical help of Mrs. VICTORIA STÜRTZ in one phase of the work is gratefully acknowledged.

E. Kovács

Deutsche Forschungsanstalt für Psychiatrie (Max-Planck-Institut), Abteilung für Serologie und Mikrobiologie, München, June 29, 1959.

Zusammenfassung

Stark cytolytisch wirkende Urea-Desoxycholatlösung zerstört die biologische Aktivität des Poliovirus (Typ I, Mahoney-Stamm) *nicht*; somit ist dieses Mittel zur Freisetzung infektiösen Materials aus latent oder manifest infizierten Zellkulturen sehr geeignet.

⁴ In preparation.

⁵ J. S. COLTER, *Nucleic Acid as Carrier of Viral Activity* in E. BERGER and J. L. MELNICK, *Progress in Medical Virology*, vol. I (S. Karger, New York-Basel 1958), p. 1.

Metabolic Interactions In Vitro between Polymorphonuclear Leukocytes and Pathogenic and Nonpathogenic Microorganisms¹

The addition of bacteria to polymorphonuclear leukocytes in the Warburg respirometer causes an alteration of the otherwise constant rate of oxygen uptake by the leukocytes. Changes of rates may be attributed to the effect exerted by bacteria on the leukocytes and by leukocytes on the phagocytized bacteria²⁻⁴. In previous studies it had been shown that tubercle bacilli maintained a constant rate of respiration in an intracellular environment as evidence of their ability to survive within phagocytic cells⁵. These investigations were extended to other pathogenic and nonpathogenic bacteria. The results of these studies are reported.

Methods. Bacteria were grown in brain-heart-infusion broth and were washed repeatedly. Peritoneal exudate leukocytes from rabbits were obtained by injecting a solution of sodium caseinate intraperitoneally 15 h prior to collection. The cells were washed twice in saline; leukocytes and bacteria were resuspended in a medium consisting of Krebs Ringer phosphate buffer containing 20% homologous, heat-inactivated serum, and 100 mg% glucose. The Warburg vessels were prepared as follows: Leukocyte suspension in main chamber, bacterial suspension (live or heat-killed bacteria) in side-arm, and 10% NaOH in center well. After equilibration in the water bath, the bacteria were added to the leukocytes by tipping the content of the side-arm into the main chamber. Manometric readings were taken every 30 min over a period of 3 to 4 h. In every experiment leukocytes alone, bacteria alone, and leukocytes with live and dead bacteria were used. Samples of mixtures and bacterial suspensions were removed from the flasks to determine number of viable bacteria and extent of phagocytosis.

Results. When oxygen uptake of leukocytes alone with that of leukocytes with *heat killed* bacteria was compared, the results represented in Table I were obtained. The average number of leukocytes per flask was 1×10^8 , and there were about 2 to 5 times as many heat killed bacteria. By dividing the rate of oxygen consumption of phagocytes with bacteria by the rate of consumption by phagocytes alone, a ratio is obtained which indicates the difference between the two sets of flasks. As can be seen, the leukocytes showed increased respiration (up to 50%) after having ingested heat killed, *nonpathogenic* bacteria, whereas a reduction of oxygen consumption occurred when the leukocytes had phagocytized heat killed, *pathogenic* organisms.

The respiration of living intracellular bacteria was calculated indirectly, assuming identical oxygen consumption by phagocytes regardless of whether or not the ingested organisms were living or dead. Including the necessary control flasks, one could obtain values for oxygen consumption by bacteria within phagocytes and by bacteria suspended in cell free media. The ratios of these two

¹ This investigation was supported by Grant E-1302 (C) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

² F. BEALL, E. LERNER, and J. VICTOR, *Amer. J. Physiol.* **168**, 680 (1952).

³ H. STÄHELIN, E. SUTER, and M. L. KARNOVSKY, *J. exp. Med.* **104**, 121 (1956).

⁴ E. H. PERKINS, F. MIYA, and S. MARCUS, *Fed. Proc.* **17**, 529 (1958).

⁵ H. STÄHELIN, M. L. KARNOVSKY, and E. SUTER, *J. exp. Med.* **104**, 137 (1956).

Table I
Effect of Phagocytosis of Heat Killed Pathogenic and Nonpathogenic Microorganisms
upon Oxygen Consumption of Polymorphonuclear Leukocytes

Organism	Pathogenicity	Ratio of respiration* leukocytes + bacteria leukocytes alone
<i>Staphylococcus epidermidis</i> , Coagulase –	nonpathogenic	1.47 ± 0.12
<i>Pneumococcus</i> II, rough	nonpathogenic	1.35 ± 0.1
<i>Pneumococcus</i> II, smooth in presence of antibody	pathogenic	1.33 ± 0.53
<i>Staphylococcus</i> , Coagulase +	pathogenic	0.78 ± 0.29
<i>Salmonella typhosa</i>	pathogenic	0.98 ± 0.02
<i>Neisseria gonorrhoeae</i>	pathogenic (not for rabbit)	0.81 ± 0.21

* Oxygen uptake in µl/h.

values are given in Table II for several species of bacteria. The respiration of *nonpathogenic* bacteria decreased rapidly upon phagocytosis, and bacterial plate counts revealed a decrease in the number of viable units in the mixture, although stainable intracellular organisms remained visible throughout the observation period. According to plate counts, *virulent* organisms remained viable within phagocytes for the entire observation period. However, three different patterns of intracellular respiratory activity were observed: (1)⁸ Intracellular respiration was extremely low with *Neisseria gonorrhoeae*. (2) Intracellular respiration was reduced up to 50% with a non-hemolytic but coagulase positive *Staphylococcus aureus* and a smooth, type II pneumococcus. (3) Respiration was increased when *Salmonella typhosa* and a hemolytic, coagulase positive *Staphylococcus aureus* were phagocytized.

Discussion. Stimulation of respiration of phagocytes during and shortly after phagocytosis has been described to occur with *Brucella* and *Mycobacterium*^{3,4}. The extent of stimulation was similar to that found in our experiments (Table I). However, in the case of nonpathogenic, heat killed organisms this stimulatory effect persisted over a prolonged period of time indicating that possibly some of the bacterial materials were available as substrate for the leukocytes. The lack of increase or even the reduction of respiration found when dead pathogenic microorganisms were phagocytized, is surprising. It is unlikely that exotoxins are responsible for this inhibition, since the organisms had been washed repeatedly and heated. The presence of endotoxin-like substances and their intracellular release is more likely, especially with *Neisseria* and *Salmonella*. The increased rate of respiration during phagocytosis of heat killed pathogenic pneumococci in

presence of antibody had been observed earlier and was found to be proportional to the amount of antibody on the surface of bacteria^{2,6}. This antibody effect can best be explained by a recent finding, that leukocytes ingest and degrade soluble antigens when combined with specific antibody⁷. Nonpathogenic organisms, as expected, ceased to respire after ingestion by phagocytes. This is also true for the virulent pneumococcus, since being an obligate extracellular parasite, it is destroyed fairly rapidly after phagocytosis⁸. Pathogenic organisms continue to respire even after having been phagocytized, some do so at an increased rate (Table II). This finding is compatible with the fact that some of these organisms, depending on their pathogenicity, are known to survive or multiply in the intracellular environment⁹. The failure of *N. gonorrhoeae* to consume oxygen within rabbit leukocytes can be ascribed to its lack of pathogenicity for the rabbit. It is interesting to note, that one of the coagulase positive staphylococci behaved like a nonpathogenic organism, whereas the other reacted as a pathogenic one. This observation that two strains of *Staphylococcus*, indistinguishable on the basis of the coagulase test, behave differently in our experiments might help in explaining some conflicting reports in regard to the ability of staphylococci to survive phagocytosis^{10,11}.

⁶ J. H. HANKS, in *Host-Parasite Relationship in Living Cells* (Charles C. Thomas, Springfield Ill. 1957), p. 69.
⁷ E. SORKIN and S. V. BOYDEN, *J. Immunol.* 82, 332 (1959).
⁸ J. R. GOODMAN, R. E. MOORE, and R. F. BAKER, *J. Bacteriol.* 72, 736 (1956).
⁹ G. FURNESS, *J. infectious Dis.* 103, 272 (1958).
¹⁰ D. E. ROGERS and R. TOMPSETT, *J. exp. Med.* 95, 209 (1952).
¹¹ Z. A. COHN and S. I. MORSE, *J. exp. Med.* 110, 419 (1959).

Table II
Oxygen Uptake of Pathogenic and Nonpathogenic Microorganisms within Leukocytes Compared with Freely Suspended Microorganisms

Organism	Pathogenicity	Ratio of respiration* bacteria within leukocytes bacteria alone
<i>Staphylococcus epidermidis</i> , Coagulase –	nonpathogenic	0.18 ± 0.12
<i>Pneumococcus</i> II, rough	nonpathogenic	0.085 ± 0.12
<i>Staphylococcus</i> , Coagulase + non hemolytic	pathogenic	0.53 ± 0.07
<i>Pneumococcus</i> II, smooth in presence of antibody	pathogenic	0.79 ± 0.08
<i>Salmonella typhosa</i>	pathogenic	1.19 ± 0.05
<i>Staphylococcus</i> , Coagulase + hemolytic	highly pathogenic	1.5 ± 0.14
<i>Neisseria gonorrhoeae</i>	pathogenic	0.18 ± 0.03

* Oxygen uptake in µl/h.

The results indicate that intracellular respiration can serve as an indicator of the outcome of the interaction between phagocytes and microorganisms and might allow rapid differentiation between obligate extracellular and facultative intracellular microorganisms¹².

J. GELZER¹³ and E. SUTER¹⁴

Department of Microbiology, College of Medicine, University of Florida, Gainesville, July 9, 1959.

Zusammenfassung

1. Es wird gezeigt, dass der Effekt von phagozytierten, hitzeabgetöteten Bakterien auf die Atmung von Kaninchen-Leukozyten *in vitro* von der Virulenz der verwendeten Mikroorganismen abhängig ist.

2. Die Tatsache, dass lebende Mikroorganismen nach Phagozytose intrazellulär fortfahren zu atmen, wird demonstriert und deren Beziehung zur Pathogenität der Bakterien diskutiert.

¹² E. SUTER, Bacteriol. Rev. 20, 94 (1956).

¹³ Present Address: University Childrens Hospital, Zurich (Switzerland).

¹⁴ Markle Scholar in Medical Science.

in two pairs of long and short chromosomes. The karyotype varies in different strains studied and structural heterozygosity in the chromosome complement was also noticed. Thus the present species differs from its European forms not only in the chromosome numbers ($2n = 30$) but also in gross chromosome types i.e. the presence of an additional pair of long chromosome with nearly median primary constriction and of a pair of short chromosome with secondary constriction (Fig. 1). Such a chromosome complement has been reported¹ to be typical of group *Alternifolia*, Baker, and so far has been unknown for the group *Verticillata*.

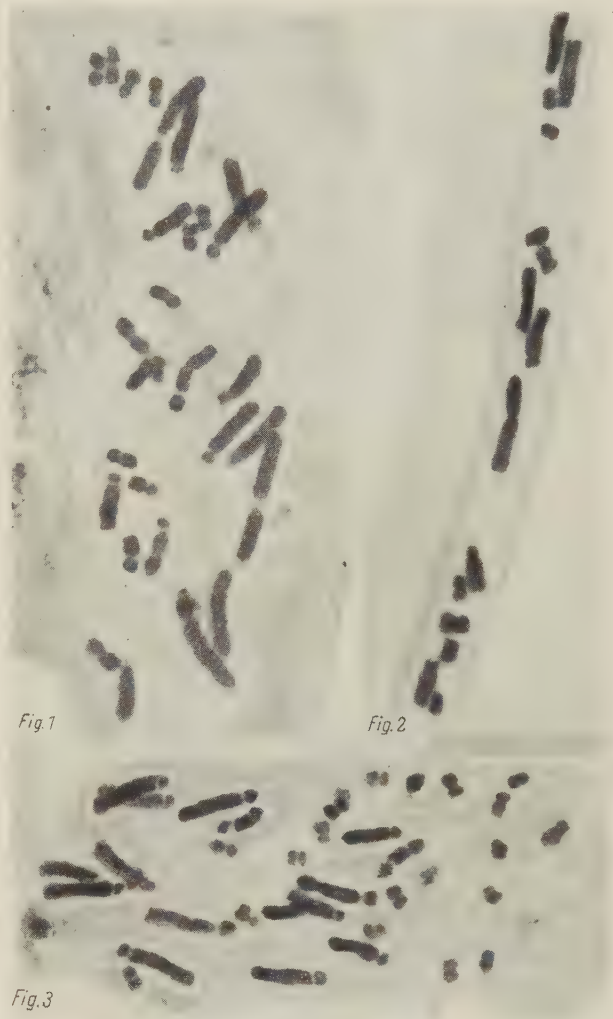


Fig. 1

Fig. 2

Fig. 3

Fig. 1.—Mitotic Stage in Root Tip Cell showing 30 Chromosomes.

Fig. 2.—The Division of the Generative nucleus in the Pollen Tube showing Haploid Chrom. Complement.

Fig. 3.—*P. cirrifolium*: Root Tip, Mitotic Stage showing 38 Chroms.

Karyotype in two Himalayan species of *Polygonatum*

The liliaceous genus *Polygonatum* is believed to have its centre of diversification in Eastern Himalayas and Western China. The present Himalayan species *P. verticillatum* Allioni and *P. cirrifolium* Royle, belong to the group *Verticillata* Baker. The former species has a wide geographic distribution; besides occurring throughout the Himalayan range, it is extended as far as Northern Europe. Extensive cytological investigations have been carried out on eighteen European strains of *P. verticillatum* and the occurrence of diploid and polyploid forms were reported^{1,2}. Though the chromosome number of all the different diploid forms studied^{1,2} was the same ($2n = 28$) differences in karyotype were observed from strain to strain, thus revealing a marked intraspecific structural heterozygosity. The karyotype reported^{1,2} for the group *Verticillata* is characterised by the presence of long chromosomes with subterminal primary constrictions and short chromosomes with subterminal and median primary constrictions. The secondary constrictions are present only in long chromosomes.

In the present study a number of strains of *P. verticillatum* and *P. cirrifolium* from Western Himalayas were analysed. The chromosome numbers, $2n = 30, 64$ in *P. verticillatum* and $2n = 38$ in *P. cirrifolium* were determined from root tip somatic plates and the division of the generative nucleus in the pollen tube (Figures 1, 2, 3). The chromosome complement of *P. verticillatum* can be broadly classified into three groups, (i) one pair of long chromosomes with nearly median primary constrictions, (ii) six pairs of medium chromosomes with subterminal primary constrictions, and (iii) eight pairs of short chromosomes with subterminal and median primary constrictions. The secondary constrictions were revealed

The karyotype of *P. cirrifolium* ($2n = 38$) is found to be allied to 'Verticillata' type² of chromosome complement as it lacks the long chromosome with sub-terminal primary constriction. However, the number of long and medium chromosomes (seven pairs) is more or less constant and also two pairs of long and short chromosomes have secondary constrictions. In this respect it is similar to the Himalayan forms of *P. verticillatum*. The karyotype of *P. cirrifolium* differs from *P. verticillatum* by the presence of four additional pairs of short chromosomes in the former.

¹ E. THERMAN, Hereditas 39, 277 (1953).

² E. THERMAN, Ann. Bot. Soc. 'Vanamo' 25, 1 (1953).

The differences in chromosome number and gross chromosome morphology between the European and Himalayan forms of *P. verticillatum* can be explained on the assumption that the Himalayan forms are primitive ones and European forms as comparatively recent ones, since it is believed that the centre of origin lies in Eastern Himalayas and Western China. Therefore, one can assume that the chromosome number $2n = 28$, noticed in the European forms has probably been derived from the original chromosome number $2n = 30$. It has been pointed out³ that the presence of long chromosomes with median constriction is a primitive feature. It is difficult to say definitely at present whether the European forms have evolved from the Himalayan ones by losing the long or short chromosome pair. However, one cannot overlook the possibility of interspecific hybridization with some species of the group *Alternifolia* and this may have subsequently been followed by introgression. A detailed study on species from Eastern Himalayas is in progress.

The author is most indebted to Dr. M. S. SWAMINATHAN, Cytogeneticist of the Indian Agricultural Research Institute, New Delhi, for his encouragement and valuable suggestions. Sincere thanks are due to Mr. M. M. BEGG, Principal of Delhi College, for providing the necessary facilities and also to the Ministry of Scientific Research and Cultural Affairs for a Grant-in-aid.

VIRENDRA KUMAR

Department of Biology, Delhi College, Delhi University, Delhi (India), June 18, 1959.

Zusammenfassung

Die Chromosomenzahl und der Karyotypus von *Polygonatum verticillatum* Allioni ($2n = 30, 64$) und *P. cirrifolium* Royle ($2n = 38$), die im Himalaya vorkommen, wurden mit früher untersuchten europäischen Formen verglichen. Die beobachteten Unterschiede lassen sich durch die Annahme erklären, dass die europäischen Formen aus den Himalaya-Formen durch den Vorgang der Chromosomenverminderung und Veränderungen des Karyotypus hervorgehen.

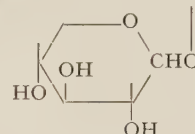
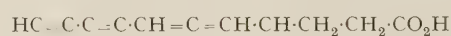
³ G. L. STEBBINS, JR., *Variation and Evolution in plants* (Oxford University Press, 1950).

Pathways of Sugar Metabolism in Relation to the Biosynthesis of Polyacetylenic Antibiotics

As part of a general programme of research into the biochemistry of antibiotic production we have been especially concerned with the Basidiomycete B-841, which produces polyacetylenic antibiotics with C_{11} (nemotinic acid, nemotin) and C_{12} (odyssic acid, odyssin) chains. Having established the structures of these compounds¹ and their biogenesis from a C_2 unit related to acetate² we turned our attention to the general metabolism of the fungus so that this might ultimately be correlated with the special processes of antibiotic formation. In this we were greatly assisted by the discovery that a high pro-

portion of the nemotinic acid produced by B-841 is in the form of the xyloside (I) (in which the stereochemistry of the allene unit, the adjacent C_4 , and the glycoside carbon atom remain uncertain). By the isolation of this compound we were enabled to study some aspects of pentose and polyacetylene synthesis simultaneously.

When B-841 is utilising glucose as sole nutrient, labelling from added $[1-^{14}C]$ acetate is efficiently incorporated (15–20%) into the polyacetylenes but not into the xylose moiety of I. However B-841 will also utilise ethanol as sole nutrient; under these circumstances both moieties are labelled by $[1-^{14}C]$ acetate, the ratio of activities in the xylose and C_{11} portions of I being about 1.5/6 (the C_{11} chain contains 6 'active' C atoms²). We conclude that in the ethanol cultures, in which all metabolic intermediates are being synthesised from C_2 units, the xylose is produced by way of compounds closely related to glucose; this explains the absence of labelling in the glucose cultures and is of course probable on general grounds.



(I); $[\alpha]_D^{25} + 237^\circ$ ($c = 0.1$ in EtOH)

Degradation of the labelled xylose revealed the pattern of labelling shown in II, the activities being relative to that of C_4 of the xylose. Our explanation of this labelling-pattern involves the operation of at least three well-known pathways of sugar metabolism in B-841.

	$CH_2OH-CHOH-CHOH-CHOH-CHO$				
Activity	27	28	45	100	3
	(II)				

Labelling from $[1-^{14}C]$ acetate is normally incorporated into sugars by way of conversion into $[1-^{14}C]$ pyruvate or its enol phosphate, followed by the reversal of Emden-Meyerhof glycolysis. We have independent evidence for the operation of the Emden-Meyerhof route and citrate cycle in B-841; in the case when ethanol is the only nutrient, a special modification of the citrate cycle must be operative. As is well known, the overall effect of this process will be the synthesis of hexose labelled equally at C_3 and C_4 . Now it has been shown that in a variety of systems the principal cause of randomisation of labelling in C_1-C_3 of hexoses is the action of enzymes of the transaldolase-transketolase cycle; the pattern of labelling in our xylose is exactly that which would be expected for C_1-C_5 of a hexose, initially labelled at C_3 and C_4 only, and subsequently randomised in this way³. This also implies that the xylose itself is not formed from one of the pentose phosphates intermediate in the transaldolase-transketolase cycle, but from a hexose derivative by loss of C_6 . Such a process is involved in a known metabolic route, viz. the conversion of glucose via glucuronic acid into pentoses, including xylose⁴.

Thus, without having isolated any of the relevant enzymes of intermediates we have circumstantial evidence

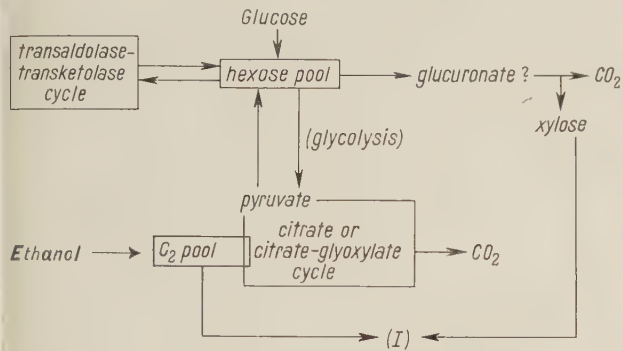
¹ J. D. BU'LOCK, E. R. H. JONES, and P. R. LEEMING, J. chem. Soc. 1955, 4270; 1957, 1097.

² J. D. BU'LOCK and H. GREGORY, Biochem. J. 72, 322 (1959).

³ H. G. WOOD and J. KATZ, J. biol. Chem. 233, 1279 (1958).

⁴ F. EISENBERG, P. G. DAYTON, and J. J. BURNS, J. biol. Chem. 234, 250 (1959).

for a substantial section of the carbon metabolism of B-841, which can be summarised as shown in the Figure.



In confirmation it may be added that more direct evidence, to be described in detail elsewhere, indicates that most, though not all, of the respiratory carbon dioxide evolved by B-841 is in fact formed by way of glycolysis (from glucose) and the citrate or citrate-glyoxylate cycle (from glucose or ethanol).

J. D. BU'LOCK and H. GREGORY

University Chemical Laboratory, Manchester, June 26, 1959.

Zusammenfassung

Der Einbau von [1-¹⁴C]-Acetat bei der Biosynthese von Xylose aus Äthanol durch die Basidiomycete B-841 beweist das Vorhandensein von drei verschiedenen Wegen des Zuckerstoffwechsels in diesem Pilz: 1. Glycolyse, 2. Transaldolase-Transketolase-Zyklus, 3. Abbau von Glucose zu Glucuronat und Pentose.

10-Hydroxy- Δ^2 -decenoic Acid in the Honeybee (*Apis mellifera*)

10-Hydroxy- Δ^2 -decenoic acid has been isolated from 'royal jelly', the food of queen larvae, and is also present in the food of worker-bee larvae¹. Its absence from nectar and pollen² suggested that it must come from the salivary glands of worker bees. Of these, the mandibular pair contain a suspension of liquid globules, soluble in sodium bicarbonate solution and reprecipitated by acids, which, in bees stored in a refrigerator, deposits masses of crystals that do not melt at room temperature³ (Fig. 1). During work on a related problem, a crystalline fraction was obtained from an extract of the heads of worker bees. The idea that both these crystalline materials were 10-hydroxy- Δ^2 -decenoic acid was supported by finding that the contents of fifty worker-bee mandibular glands yielded by solvent extraction and fractionation a crystalline acid, which by the criteria of infrared absorption, rate of flow on a paper chromatogram and rate of travel in a paper electrophoresis, was identical with the material extracted from worker-bees' heads and with authentic 10-hydroxy- Δ^2 -decenoic acid extracted by the method of BUTENANDT

¹ A. BUTENANDT and H. REMBOLD, Hoppe-Seylers Z. 308, 204 (1957).

² S. A. BARKER, A. B. FOSTER, D. C. LAMB, and N. HODGSON, Nature 183, 996 (1959).

³ J. SIMPSON, J. ins. Physiol. 4, (1960), in press.

and REMBOLD from 'royal jelly' ¹. Proof that the crystals seen in the contents of a mandibular gland after freezing were identical with 10-hydroxy- Δ^2 -decenoic acid was obtained by X-ray powder photographs (Fig. 2).



Fig. 1.—Crystals from mandibular gland of the honeybee. Crossed Polaroid. $\times 540$.

It is thus clearly indicated that the 10-hydroxy- Δ^2 -decenoic acid in 'royal jelly' comes from the mandibular glands of the worker bee. The contents of these glands contain little protein⁴, so the belief, based on strong cir-

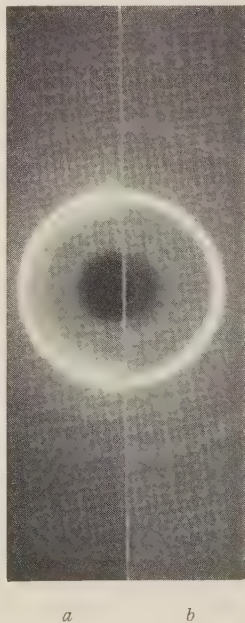


Fig. 2.—X-ray powder photographs
(a) Crystals from mandibular gland.
(b) 10-Hydroxy- Δ^2 -decenoic acid extracted from royal jelly.

⁴ E. KRATKY, Z. wiss. Zool. 139, 120 (1931).

cumstantial evidence⁵, that the hypopharyngeal glands supply the protein in larval food needs no revision. The presence of mandibular gland secretion in the food of worker larvae diminishes the likelihood that these glands are the source of the substance^{6,7} that causes female larvae to develop as queens.

Fifty mandibular glands, ground with sand, were extracted with ether and the ethereal extract (2 mg) worked up by the method of BUTENANDT and REMBOLD¹. The strong-acid fraction weighed 1.4 mg (A).

Worker-bees' heads were extracted for another purpose and the yield cannot be stated exactly. From a portion of an alcoholic extract of 1830 heads an acidic fraction was obtained. This was partitioned between ethanol and light petroleum. The ethanol-soluble fraction on a reverse-phase paper chromatogram (kerosene-30% aqueous *n*-propanol) gave a spot which, eluted with ethanol, yielded crystalline material (B).

About 1 g of royal jelly yielded 22 mg crystalline strong-acid fraction (C), m. p. 45–51°C.

Materials (A), (B), and (C), incorporated in KCl plates, gave essentially identical infra-red absorption spectra (Perkin-Elmer double-beam instrument, Model 21, NaCl prism) and the absorption bands agreed with the descriptions in the literature^{1,2}. When chromatographed on Whatman No. 1 paper with the Bush system toluene: ethyl acetate: methanol: water: 9:1:5:5, materials (A), (B), and (C) gave spots at the same distance from the origin, *R_f* 0.50 and, in the Bush system, toluene: methanol: water: 4:3:1, spots at the same distance from the origin, *R_f* 0.20. Paper electrophoresis in 0.1 *M* phosphate buffer, pH 8 gave spots moving at the same rate with (A), (B), and (C).

Dr. OLGA KENNARD very kindly took the X-ray powder photographs in Figure 2. The left side (a) is of the material from a mandibular gland, and right side (b) is of authentic material from royal jelly. Coincidence of the principal lines indicates identity of the two materials, but the glandular material appears to be somewhat contaminated.

We are indebted to Mr. M. YOUNG for the photomicrograph in Figure 1.

R. K. CALLOW, N. C. JOHNSTON, and J. SIMPSON

National Institute for Medical Research, Mill Hill, London and Rothamsted Experimental Station, Harpenden (Herts.), July 13, 1959.

Zusammenfassung

Die Mandibulardrüsen der Honigbienen-Arbeiterinnen enthalten 10-Hydroxy-Δ²-decensäure. Es wird angenommen, dass diese Drüsen die Quelle der im Weisel- und Arbeiterinnenlarvenfutter vorkommenden 10-Hydroxy-Δ²-decensäure sind.

⁵ Reviewed by C. R. RIBBANDS in *The Behaviour and Social Life of the Honeybee* (Bee Research Association, London 1953).

⁶ W. VON RHEIN, Arch. Entw. Mech. Org. 129, 601 (1933).

⁷ N. WEAVER, Science 121, 509 (1955).

Über die Grenzen der metabolischen Adaptationsfähigkeit von *Aspergillus niger*

Nach einer früheren Mitteilung¹ vermag das «Succinodehydrasegift» Malonsäure den Abbau von Zitronen- bzw.

¹ K. TÄUFEL und H. RUTTLOFF, Exper. 14, 276 (1958). – Vgl. auch K. TÄUFEL und H. RUTTLOFF, Nahrung 3 (1959), im Druck.

Tabelle

Einwirkung von fertigen Myzeldecken von *A. niger* auf Malonsäure bzw. verschiedene Derivate ohne bzw. mit Zusatz von 3% Zitronensäure (dargestellt anhand chromatographischer Untersuchungen)

Säurekomponente \ Konzentration	kein Zitronensäurezusatz		Zitronensäurezusatz			
	10 ⁻¹ M/l	3·10 ⁻¹ M/l	10 ⁻¹ M/l	Zitronensäure M/l	3·10 ⁻¹ M/l	Zitronensäure
1	2	3	4	5	6	7
Malonsäure	+	+	+	+	+	+
Methylmalonsäure	+	+	+	+	+	–
Äthylmalonsäure	(+)	(+)	(+)	+	–	–
Propylmalonsäure	–	–	–	–	–	–
Dimethylmalonsäure	–	–	–	+	–	–
Diäthylmalonsäure	–	–	–	(+)	–	–

Erläuterungen: + Säure wird dissimiliert.
(+) Säure wird wenig dissimiliert.
– Säure wird nicht dissimiliert.
+* Säure wird in Gegenwart von Zitronensäure beschleunigt dissimiliert.

Bernsteinsäure durch *A. niger* nicht nur nicht zu hemmen, sondern es wird selbst – zum Beispiel einer vorgebildeten Myzeldecke als einzige C-Quelle dargeboten – weitgehend metabolisiert. Bei Oxalsäure erfolgt der Umsatz nur in Gegenwart einer zusätzlichen dissimilierbaren Kohlenstoffverbindung. Wir deuteten dies mit einer Decarboxylierung der Malon- zu Essigsäure und deren stoffwechselchemischer Einbeziehung entweder in den Zitronensäurezyklus (Energiegewinnung) oder in den Glyoxylsäurezyklus (Bildung von Kohlenhydrat bzw. zelleigener Substanz).

Der Befund der metabolischen Verwertung von Malonsäure durch *A. niger* legt die Frage nahe, ob und inwieweit Derivate der Malonsäure mit Seitenkette, zum Beispiel ihre Mono- und Dialkylverbindungen, in den Stoffwechsel einbezogen werden. Mit dieser Zielsetzung wurden die Methyl-, Dimethyl-, Äthyl-, Diäthyl- und Propylsubstitutionsprodukte der Malonsäure allein wie in Gegenwart von Zitronensäure «fertigen» Myzeldecken von *A. niger* angeboten.

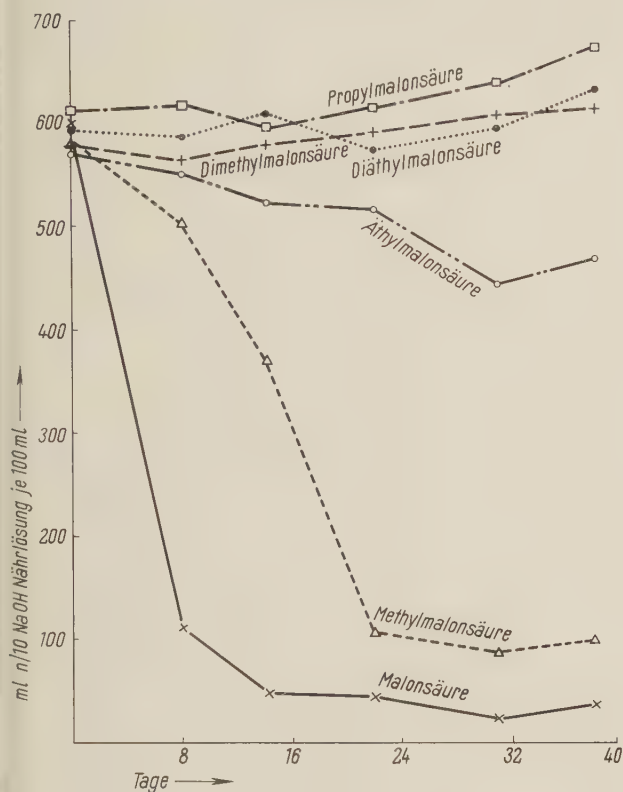
Präparate und Methoden. Die Darstellung der Malonsäurederivate erfolgt in der üblichen Weise mittels Alkyljodid über die Mono- bzw. Dinatriumverbindungen der Ester unter nachfolgender Verseifung².

Die mikrobiologische Technik bedient sich des Prinzips der Kultur der fertigen Pilzdecken¹ auf einer Nährlösung nach H. HAEHN (Saccharosebasis). Die vorgebildeten Myzelien (jeweils 5 Parallelansätze) werden auf die zur Untersuchung vorgesehene Nährlösung (nach HAEHN) übertragen, wobei statt der Saccharose nunmehr das zu testende Substrat als Kohlenstoffquelle eingesetzt wird; eine pH-Einstellung erfolgt nicht. Anschliessend werden die Ansätze bei 30°C bebrütet; über experimentelle Einzelheiten unterrichtet die Tabelle.

Analytik. In bestimmten Zeitabständen (0, 5, 10, 15, 25, 30 Tage) werden jeweils etwa 3 ml der Untersuchungslösung steril entnommen. Man titriert 2 ml hiervon nach

² L. GATTERMANN und H. WIELAND, *Die Praxis des organischen Chemikers*, 33. Aufl. (Verlag Walter de Gruyter & Co., Berlin 1948), S. 229; *Beilstein's Handbuch der Organischen Chemie*, Bd. II, 4. Aufl. (Verlag J. Springer, Berlin 1920), S. 648. – K. WINTERFELD, *Praktikum der organisch-präparativen pharmazeutischen Chemie*, 3. Aufl. (Verlag Th. Steinkopff, Dresden und Leipzig 1950), S. 87, 155.

entsprechender Verdünnung mit destilliertem Wasser mit 0,1 *n* Natronlauge gegen Phenolphthalein; die Kurven in der Abbildung stellen den Verbrauch an ml 0,1 *n* Natronlauge je 100 ml Lösung dar. Der andere Teil der Probe



Einwirkung von vorgebildeten Myzeldecken aus *A. niger* auf $3 \cdot 10^{-1}$ M Lösungen von Malonsäure bzw. Derivaten derselben (Nährsalze nach H. HAEHN)

wird chromatographisch geprüft. Man trägt jeweils 5×4 mm³ bzw. 10×4 mm³ nacheinander auf das Papier auf und entwickelt absteigend mit Butanol/Ameisensäure (100:15, mit Wasser gesättigt); als Sprühmittel dient Bromphenolblau (0,05%ige alkoholische Lösung, der zu je 100 ml Lösung 3 ml 0,1 *n* NaOH zugesetzt sind); man erhält gelbe Flecken auf blauem Grund.

Auswertung der Ergebnisse. Gemäss Abbildung wird die Malonsäure am raschesten, die Methylmalonsäure etwas verzögert dissimiliert; das Äthylderivat ist nur noch sehr wenig, die Propylverbindung praktisch nicht mehr umsetzbar. Die geprüften dialkylierten Malonsäuren sind offensichtlich für den Pilz nicht verwertbar. Das gleiche Verhalten zeigt sich, wenn man die Säuren in geringerer Menge darbietet (10^{-1} M). Das bei einigen Kurven in der Abbildung manifest werdende leichte Ansteigen des Säuregehaltes mit voranschreitendem Gärverlauf beruht auf der experimentell kaum ausschaltbaren Wasserverdunstung der Proben. Die chromatographischen Ergebnisse (Tabelle) stehen mit den Befunden nach der Abbildung im Einklang.

Zugabe von Zitronensäure erhöht nach der Tabelle, Spalte 4, die Abbaugeschwindigkeit der getesteten Säuren lediglich im Falle der Malon- sowie der Methylmalonsäure, allerdings nur in geringem Umfang. Entgegen dem Verhalten der Oxalsäure tritt eine Dissimilation der für sich allein nicht abbaufähigen Verbindungen (Propyl-, Dimethyl-, Diäthylmalonsäure) auch in Gegenwart von Zitronensäure nicht in Erscheinung (Tabelle, Spalte 6);

bei geringer Substratkonzentration, zum Beispiel bei 10^{-1} M/l Dimethyl- bzw. Diäthylmalonsäure, wird ausschliesslich die zugesetzte Zitronensäure dissimiliert; letztere bleibt unverwertbar liegen, wenn grössere Mengen am Disubstitutionsprodukt (zum Beispiel $3 \cdot 10^{-1}$ M/l) vorhanden sind (Tabelle, Spalte 7).

Zusammenfassend ergibt sich, dass bei Monosubstituierung der von *A. niger* verwertbaren Malonsäure mit zunehmender Kohlenstoffzahl ihre Abbaufähigkeit abnimmt. Unterstellt man für die metabolische Einbeziehung der Malonsäuren eine Decarboxylierung, so nimmt die Fähigkeit des Mikroorganismus hierzu mit steigender Kettenlänge der Alkyle ab. Nach neueren Untersuchungen³ spielt die Methylmalonsäure – über eine Carboxymethylmalonsäure – im tierischen Organismus beim Abbau von Propionsäure als Durchgangsglied auf dem Wege zu Bernsteinsäure eine Rolle. Das Einmünden in den Zitronensäurezyklus könnte ihre rasche Dissimilation auch bei *A. niger* erklären. Eine im gleichen Sinne ablaufende Reaktionsfolge bzw. eine Decarboxylierung erscheint bei Äthylmalonsäure nur noch beschränkt, bei Propylmalonsäure nicht mehr möglich.

Dialkylierte Malonsäurederivate sind – auch in Gegenwart von Zitronensäure – für das Myzel nicht verwertbar; dem Pilz stehen offenbar die erforderlichen Enzyme nicht zur Verfügung. Es ist offenzulassen, ob die Fähigkeit zum Umsatz solcher «unphysiologischer» Verbindungen durch gesteuerte Adaptation realisierbar ist.

K. TÄUFEL und H. RUTTLÖFF

Deutsche Akademie der Wissenschaften zu Berlin, Institut für Ernährung in Potsdam-Rehbrücke, 8. Mai 1959.

Summary

The ability of *A. niger* to assimilate malonic acid, which we consider to be the way both of obtaining energy (citric acid cycle) and of synthesizing cell substances (glyoxylic acid cycle), is reduced by substitution. While methyl malonic acid is quickly assimilated and ethyl malonic acid more slowly propyl-, dimethyl-, and diethyl malonic acid cannot be used by already formed molds.

³ Vgl. Bericht in Chemiker-Ztg. 80, 204 (1956).

The *in vivo* Effect of some Peroxides upon the Mouse Ascites Carcinoma of Ehrlich¹

It has been suggested that some of the biological events following the passage of radiation through living tissue may be due to formation of peroxides^{2,3}. Because of the well known palliative effect of irradiation in certain malignancies, the survival of mice (white Swiss, weight about 25 g) inoculated with ascites tumor cells (20×10^6 cells) was investigated after intraperitoneal injections of various peroxides 24 h after implantation of the tumor cells.

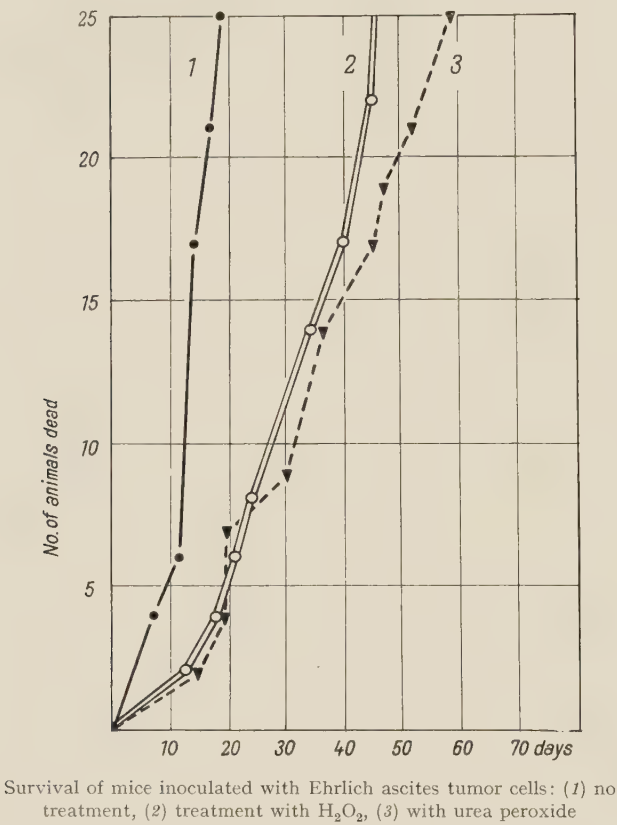
Hydrogen peroxide and urea peroxide (Amend, New York) prolonged the life span of the mice inoculated with

¹ This work was supported by a grant from the United States Public Health Service (C-3538).

² M. R. ZELLE in A. HOLLAENDER, *Radiation Biology* (McGraw-Hill Book Company, 1955).

³ V. J. HORGAN, J. ST. PHILPOT, BARBARA W. PORTER, and D. B. ROODYN, *Biochem. J.* 67, 551 (1957).

these tumor cells considerably; mean survival was increased from about 12 to about 30 and 35 days respectively, as can be seen from a representative experiment in the Figure. (Three such experiments were carried out.) The dose schedule was as follows: H_2O_2 360 mg/kg in 1 cm³ water daily for seven days; urea peroxide 800 mg/kg in 1 cm³ water daily for two days followed by 400 mg/kg daily for four days. Hydrogen peroxide has already been tested by CHORAŽY *et al.*⁴ and SUGUIRA⁵ and found to have no effect on the Ehrlich ascites tumor cells; however, CHORAŽY *et al.* administered it in drinking water, whereas the dose used by SUGUIRA (150 mg/kg) was far lower than ours.



Most of the mice treated with these compounds were autopsied; interestingly, all showed solid tumors. Four specimens of these specimens, kindly examined by Dr. G. H. FRIEDEL (Massachusetts Memorial Hospital), showed essentially identical findings. One report reads as follows: 'Compatible with Ehrlich ascites tumor. There are extensive areas of necrosis. Stroma is minimal in amount. Tumor is infiltrating muscle and shows perineural and perivascular lymphatic invasion.'

The following compounds did not prolong survival as the treated animals showed well developed ascites tumors similar to the controls: Hydroxyheptyl peroxide (Lucidol, Buffalo, New York, 20 mg/kg in 0.05 cm³ ethanol daily for two days), *p*-menthane hydroxperoxide (Lucidol, 400 mg/kg in 0.5 cm³ mineral oil daily for three days), dicumyl peroxide (G. L. Cabot, Cambridge, Massachusetts, 1000 mg/kg in 0.5 cm³ mineral oil once), succinic

acid peroxide (Lucidol, 80 mg/kg for five days in 1 cm³ water). We are presently engaged in an extensive study of the effect of peroxide compounds of different stability on various experimental tumors.

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R. R. CAMPBELL

Department of Medicine, Boston University School of
Medicine, Boston (Mass.), June 2, 1959.

Zusammenfassung

Die Lebensdauer von mit Ehrlich-Ascites-Karzinom injizierten Mäusen wird durch Wasserstoffsuperoxid und Harnstoffperoxyd auffallend verlängert. Der Tumor wird dabei von der Ascites- in die solide Form umgewandelt.

An Antagonism Between 3-4 Dioxypyhenylalanine
(DOPA) and 5-Hydroxytryptophan
(5-HTP)

Recently, it has been suggested that 5-hydroxytryptamine and norepinephrine may be mediators at the C.N.S. level. In connection with a previous theory of HESS¹, BRODIE² suggested that the two opposing subcortical systems, integrating autonomic somatic and psychic functions, might be regulated by serotonin (trophotropic system) and norepinephrine (ergotropic system). There is also some evidence that an antagonism between serotonin and norepinephrine occurs both *in vitro*³⁻⁵ and *in vivo*^{3,6}.

Table I

Treatment	No. of animals	Sleeping time (min)	P-value
(1) Hexobarbital (H)	20	33 ± 2.9	
(2) 5-HTP + H. . .	20	54 ± 3.4	(1)-(2) 0.001 > P
(3) DOPA + H. . .	20	32 ± 3.5	(4)-(2) 0.001 > P
(4) 5-HTP + DOPA + H.	20	34 ± 3.3	(1)-(3) N. S.
			(4)-(3) N. S.

5-HTP 50 mg/kg and DOPA 50 mg/kg were injected intraperitoneally 30 min before the injection of hexobarbital 80 mg/kg i. p. Room temperature (18-19° C)

The purpose of the experiments reported here was to investigate whether or not norepinephrine could antagonize the potentiation of barbiturate narcosis induced by

¹ W. R. HESS, *Das Zwischenhirn* (Benno Schwabe and Co., Basle 1954).
² B. B. BRODIE and P. A. SHORE, *Ann. N. Y. Acad. Sci.* 66, 631 (1957).
³ R. JAQUES, H. J. BEIN, and R. MEIER, *Helv. physiol. Acta* 14, 269 (1956).
⁴ S. GARATTINI and L. VALZELLI, *Boll. Soc. ital. Biol. sper.* 32, 288 (1956).
⁵ S. GARATTINI and L. VALZELLI, *Boll. Soc. ital. Biol. sper.* 32, 292 (1956).
⁶ P. GORDON, F. J. NADDY, and M. A. LIPTON, *Science* 128, 531 (1958).

⁴ M. CHORAŽY, A. GETTLICH, L. GÓRAL, B. KOŁOCZEK, E. MOŁAWKA, B. PENAR, and Z. SZWEDA, *Nature* 182, 394 (1958).
⁵ K. SUGUIRA, *Ann. N. Y. Acad. Sci.* 76, 575 (1958).

serotonin^{5,7,8}. Since serotonin and norepinephrine penetrate the blood-brain barrier with difficulty, their precursors, DL-5-hydroxy-tryptophan (5-HTP, California Biochemical Research) and DL-2,4-dioxyphenylalanine (DOPA-Hoffmann-La Roche), were used.

Table II

Treatment	No. of animal	Sleeping time (min)	P-value
(1) Pentobarbital P.	18	33 ± 2.9	
(2) 5-HTP + P.	18	50 ± 3.7	(1)–(2) 0.01 > P > 0.001
(3) DOPA + P.	18	36 ± 2.5	(4)–(2) 0.05 > P > 0.02
(4) 5-HTP + DOPA + P.	18	39 ± 3.0	(1)–(3) N. S. (4)–(3) N. S.

5-HTP 50 mg/kg and DOPA 20 mg/kg were injected intraperitoneally 30 min before the injection of pentobarbital 50 mg/kg. Room temperature (22–23° C)

Female swiss mice of the average weight of 20 g were injected i. p. with 5-HTP or DOPA (dissolved in distilled water) 30 min before the administration of pentobarbital (50 mg/kg i. p.) or hexobarbital (80 mg/kg i. p.). The sleeping time was determined, by observing the duration of the loss of righting reflex.

The results obtained are summarized in Tables I and II.

It was observed that 5-HTP potentiates both pentobarbital and hexobarbital. On the contrary DOPA, at the concentration tested, did not affect the barbiturate sleeping time, but completely reversed the potentiation induced by 5-HTP. These results do not necessarily imply an antagonism between norepinephrine and serotonin. Recent data showed that DOPA and 5-HTP compete for the decarboxylase enzyme(s)⁹. Assuming that serotonin is responsible for the barbiturate increased activity, the observed antagonism could be interpreted as a decrease in the formation of serotonin in the presence of DOPA. On the other hand, if serotonin potentiates barbiturate action by a peripheral effect, the antagonism observed with DOPA could be explained on the basis of the known antagonism between serotonin and norepinephrine¹⁰.

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Istituto di Farmacologia e di Terapia, Università degli Studi, Milano, July 10, 1959.

Riassunto

Recentemente è stata formulata l'ipotesi che la 5-idrossitriptamina e la noradrenalina agiscano come mediatori a livello del S.N.C. I risultati ottenuti dall'A. dimostrano che la diossifenilalanina può antagonizzare il potenziamento della narcosi barbiturica indotto dal 5-idrossitriptafano.

⁷ P. FARNAROLI and M. KOLLER, *Il Farmaco*, ed. sci. 9, 546 (1954).
⁸ F. N. FASTIER, *Exper.* 12, 351 (1956).
⁹ A. YUWIBER, E. GELLER, and S. EDUSON, *Arch. Biochem. Biophys.* 80, 162 (1958).
¹⁰ It should be also recalled that a potentiation between serotonin and norepinephrine has been observed ¹¹.
¹¹ R. MEIER, T. TRIPOD, and E. WIRZ, *Arch. int. Pharmacodyn* 109, 55 (1957).

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Immunological Unresponsiveness in Chickens Induced by Human γ-Globulin

Many authors¹ have reported the supression of the immune response in fetal and new-born rabbits due to excessive amounts of foreign protein antigens injected into animals. Recently, WOLFE *et al.*² succeeded in producing immunological unresponsiveness in newly hatched chickens by injecting them with bovine serum albumin. The present report deals with the antibody production in chickens following injections of human γ-globulin into embryos and infant animals.

Rhode Island Red embryos and chicks were used in the experiments. Chicken embryos of Group I were intravenously injected on the 12th day of incubation with 16 mg of human γ-globulin (HGG), followed by a rest until the sixth week of age. Infant chicks of Group II received subcutaneously eight injections of HGG (16 mg/injection). First injection was given one day after hatching and other injections in four-day intervals until the 32nd day of postnatal life. Group III of uninjected chicks served as control. When the experimental animals of all groups were 42 days old, they were inoculated with 40 mg of HGG. Control bleedings were performed in the sixth week of age prior to the injection of 40 mg of HGG, and the test bleedings seven days after HGG had been administered. All sera were tested on the same day to avoid variation in titers³. The animals of the Group II and Group III were further treated with HGG (40 mg/injection). The animals were hatched and maintained in our laboratory.

Anti-human globulin antibody in chicken sera was determined by Coombs anti-human globulin technique. Red cells from a single donor of group A, cDE/cDE were used. A 5-% suspension was prepared in 10 ml of an anti-D+E serum diluted 1:50. The same antiserum and the same dilution was used throughout the experiment. The incubation was 60 min at 37°C. The sensitized cells were washed three times in saline and a 2-% suspension was prepared. The chicken sera were made up in serial doubling dilutions and distributed in 0.05 volume in tubes (7×45 mm). To each tube 0.05 ml of sensitized red cell suspension was added and allowed to stand for 2 h at 37°C. The results were read microscopically on the slide. Parallel series of diluted chicken sera were set up for the determination of heteroagglutinins. Nonsensitized A, cDE/cDE red cells were used as antigen.

The Table records the results obtained with chicken sera after the injection of 40 mg of HGG. Control bleeding sera of all three groups of chicks, obtained prior to the injection of 40 mg of HGG, were also tested, using both sensitized and nonsensitized red cells. But they have shown only the activity of hetero-agglutinins and not the activity of anti-human globulin antibody.

These results indicate that chickens which received several injections of HGG in the early period of postnatal life produced antibody at a much lower level, when assayed on the 7th day after the challenging injection. In the 13th week, the antibody production reached the level of the control group, tested in the seventh week of age.

¹ R. HANAN and J. OYAMA, *J. Immunol.* 73, 49 (1954). – F. J. DIXON and P. H. MAURER, *J. exp. Med.* 101, 245 (1955). – B. CINA-DER and J. M. DUBERT, *Brit. J. exp. Path.* 36, 515 (1955). – R. T. SMITH and R. A. BRIDGES, *Transpl. Bull.* 3, 145 (1956).
² H. R. WOLFE, C. TEMPELIS, A. MUELLER, and S. REIBEL, *J. Immunol.* 79, 147 (1957). – C. H. TEMPELIS, H. R. WOLFE, and A. MUELLER, *Brit. J. exp. Path.* 39, 323, 328 (1958).
³ N. GENGOZIAN and H. R. WOLFE, *J. Immunol.* 78, 401 (1957)

Preliminary treatment	No. of chick	Age, 6 weeks	Age, 7 weeks		Age, 13 weeks	
			Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Group I Single i.v. injection of 16 mg HGG (12-days embryos)	10	One injection of 40 mg HGG	64-512	217,6 $\pm$ 50,25		
Group II Eight s. c. injection of 16 mg of HGG each at age 1-32 days	10	One injection of 40 mg HGG	16-64	40 $\pm$ 6,54	*64-512	234 $\pm$ 52,7
Group III None (control)	12	One injection of 40 mg HGG	64-512	213,3 $\pm$ 45,86	128-1024	298 $\pm$ 72,33

* Nine chicks tested

A single injection of antigen given to 12-days old embryos had no effect on the latter immune response. This data seems to support the view of SIMONSEN⁴ that the day of injection may be of significance for reduction of immunological responsiveness.

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Microbiological Institute, Belgrade University, School of Pharmacy, Belgrade (Yugoslavia), May 8, 1959.

Zusammenfassung

Wiederholte Injektionen von menschlichem γ -Globulin in der ersten Nachgeburtsperiode, führte bei Kücken zu immunologischer Toleranz gegenüber diesem Antigen.

⁴ M. SIMONSEN, Acta path. microbiol. scand. 39, 21 (1956).

Cholinesterase in Human Salivary Glands

In case of tumour in or near the salivary glands, surgical removal of the tumour and surrounding glandular tissue is the standard treatment. Advantage of this fact was taken to obtain fresh human salivary gland tissue for estimation of cholinesterase activity. Previously, the cat submaxillary and parotid gland (STRÖMBLAD¹) have been found to contain cholinesterase.

Methods. Only apparently healthy tissue was used; the tumour was cut off with a wide margin. The tissue was carefully cleaned, washed in saline, weighed, minced with scissors, and ground in a glass homogenizer with Krebs' bicarbonate-Ringer. The volume in ml was then brought up to five times the weight of the tissue in g.

The cholinesterase estimations were made manometrically using Warburg flasks of conventional shape and size. The main compartment contained 0.2 ml of the homogenate and 1.5 ml of Krebs' bicarbonate-Ringer solution. The substrates, in the side bulb in 0.3 ml, were acetylcholine, methacholine, or benzoylcholine in concentrations giving final reaction molarities of 0.011, 0.035, and 0.290, respectively. The substrates were added after

15 min of temperature equilibration. The gas was N₂ with 5% CO₂ and the temperature 37° C. Readings were taken every 5 min for 40 min. In all experiments, a thermobarometer and enzyme blank were used. Corrections were made for changes in these flasks and for non-enzymic hydrolysis. All estimations were made in duplicate. The activity was expressed in μ l CO₂ evolved/30 min/g of tissue.

In some experiments, a small volume of eserine salicylate giving a reaction mixture molarity of 3.63×10^{-6} was tipped into the main chamber 25 min after estimation had begun. The CO₂ evolution was almost completely abolished and this was taken as evidence that the reactions studied were enzymic breakdown of choline esters.

Table

	Acetylcholine	Methacholine	Benzoylcholine
Parotid glands	♂ 1335 $\pm$ 97.7 (n = 8)	493 $\pm$ 49 (n = 7)	75 $\pm$ 233 (n = 6)
	♀ 1035 $\pm$ 64.8 (n = 8)	405 $\pm$ 30 (n = 5)	70 $\pm$ 20.0 (n = 5)
Submaxillary glands	♂ 1463 $\pm$ 29.8 (n = 6)	573 $\pm$ 28.6 (n = 5)	133 $\pm$ 26.9 (n = 5)
	♀ 1183 (n = 3)	388 (n = 3)	50 (n = 3)

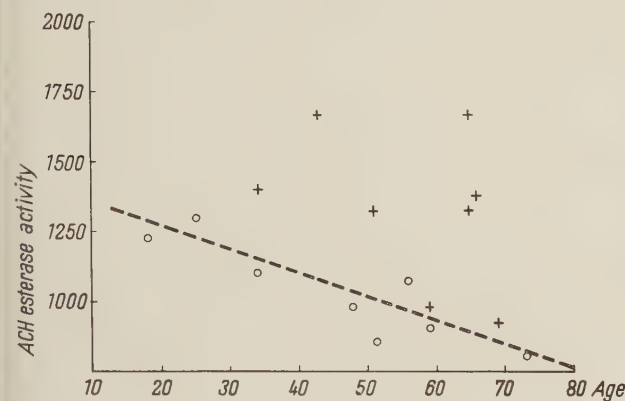
Results and Discussion. The human parotid and submaxillary glands showed a moderately high cholinesterase activity (see Table). The activity with acetylcholine and methacholine was much higher than with benzoylcholine, in fact in some cases no enzymic splitting of benzoylcholine was found. The substrate concentration-activity curve showed an almost regular bell-shaped form. Thus the cholinesterase in the human salivary glands is mainly true cholinesterase (acetylcholine esterase). The same is true for cat glands (STRÖMBLAD¹).

The acetylcholine splitting activity was higher for male parotids than for female parotids when analyzed with Student's *t*-test ($P < 0.05$). When the acetylcholine-splitting activity of male and female glands was plotted against the age of the patients, a correlation was found for females between age and activity ($r = 0.807$, $P < 0.01$). No such close correlation could be demonstrated for the male parotid glands. Analysis of variance

¹ B. C. R. STRÖMBLAD, Acta physiol. scand. 41, 118 (1957).

was applied and the difference between males and females was then significant on the level of $P < 0.01$.

LACASSAGNE² found histological differences between the male and female submaxillary glands from rats. He was also able to change the appearance of the structures by injections of sex hormones. It is therefore possible that the difference in enzymic activity in human male and female parotid glands is due to sex hormonal influence.



The Figure shows the acetylcholine splitting activity (ordinate) plotted against the age (abscissa) for male (+) and female (o) parotids. The regression line for female glands ($k = -0.334$) is drawn.

Since usually only a small part of the gland was obtainable, it was thought of interest to know whether any differences found between glands could be due to differences in activity in different parts of the gland. This possibility was tested in cases where much tissue was at disposal. The tissue was divided into several pieces and the activity of each estimated. It was found that the results obtained with different pieces from one gland were very similar. One, almost whole, parotid gland, for example, was divided into 16 parts and the acetylcholine splitting activity was estimated. The standard error in this series was $\pm 2.1\%$, that is of an order which might be due to the error of the method used. Thus the cholinesterase in parotid glands seems to be evenly distributed. The activity was about the same in human parotid and submaxillary glands (see Table). In the cat, the activity in the submaxillary gland is about double that of the parotid gland (STRÖMBLAD¹).

B. C. R. STRÖMBLAD

Institute of Physiology, University of Lund (Sweden), May 25, 1959.

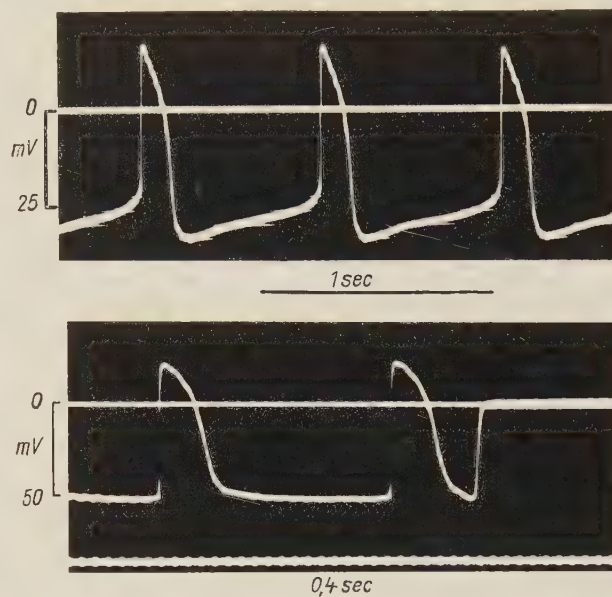
Zusammenfassung

Operativ frisch entnommene menschliche Speicheldrüsen (Submaxillaris und Parotis) zeigen Cholinesteraseaktivität. Hauptsächlich werden spezifische Esterasen gefunden. Die Aktivität der männlichen Parotis ist höher als die der weiblichen. Letztere zeigt eine Aktivitätsabnahme mit zunehmendem Alter.

² A. LACASSAGNE, C. R. Soc. Biol. 133, 180 (1940).

Early Functional Differentiation of Heart Muscle Cells¹

Tissue cultures from heart muscle cells contract spontaneously. According to FÄNGE *et al.*², all cells show an electrical behaviour characteristic of pacemaker regions, the so-called prepotentials. The pacemaker properties are evident even if the material is taken from parts of the embryonic heart that would differentiate into ventricular tissue, provided the embryos are younger than 192 h (8 days). The question arises whether *in vivo* all heart fibres at an early stage of differentiation show pacemaker activity³.



Upper record: transmembrane potential of sinus region of a 42-h old chick embryo. Lower record: transmembrane potential of cardiac tube (future ventricle)

Fertile chick eggs, incubated for 37 to 67 h at 38°C, were used. The yolk was removed by suction under warm Locke's solution. The embryo, with intact area vasculosa, was transferred to a Perspex chamber. This contained Locke's solution thermostatically controlled at 37°C and aerated with oxygen (95%) and CO₂ (5%). With the help of a binocular microscope, the membranes overlying the heart were dissected, and a single muscle fibre was impaled with a glass capillary microelectrode (tip $< 0.5 \mu$).

The potential difference between the microelectrode and an indifferent electrode was applied to a cathode-follower with a low grid current⁴, amplified, displayed on a cathode ray tube and recorded on moving film. The second beam of a dual beam scope was used to indicate the level of zero potential difference. Embryos from successful experiments were kept in order to check their age and to measure the diameter of their cardiac fibres.

¹ Aided by a grant from the Italian 'Consiglio Nazionale delle Ricerche'.

² R. FÄNGE, H. PERSSON, and S. THESLEFF, Acta Physiol. Scand 38, 173 (1957).

³ See also the comparative physiological researches on the isolated embryonic fishheart by H.J. HUGGEL (Z. vgl. Physiol. 42, 63 (1959)), which showed by the ligaturemethod the early development of pacemaker activity.

⁴ E. MEDA, Arch. Fisiologia 58, 404 (1958).

Amplitude of Prepotential, Membrane Potential and Overshoot of Embryonic Chick Sinus Venosus and Ventricle (37 C).

n = number of measurements; $\bar{m}$ = mean; σ = standard deviation

Exp. No.	Age in h	So. mites	Prepotential mV (n) $\bar{m} \pm \sigma$ (range)	Membrane resting potential mV (n) $\bar{m} \pm \sigma$ (range)	Overshoot mV $m \pm \sigma$ (range)	Action Pot. durat. sec. $\bar{m} \pm \sigma$	Mean heart rate per min.
1	37	9	Ventr. { Sin. (41) 8.3 $\pm$ 1.49 (3.8-11.1)	(7) 51.6 $\pm$ 0.00 (51.0-53.0) 33.3 $\pm$ 8.38 (19.1-41.0)	20.5 $\pm$ 0.46 (12.8-21.2) 20.2 $\pm$ 6.55 (9.5-27.7)	0.214 $\pm$ 0.01 0.193 $\pm$ 0.09	130 124
2	42	13	Ventr. { Sin. (80) 6.7 $\pm$ 1.95 (4.1-11.0)	(28) 31.0 $\pm$ 1.42 (27.5-33.9) 37.6 $\pm$ 7.46 (28.8-51.0)	25.7 $\pm$ 1.67 (21.3-27.6) 11.3 $\pm$ 5.00 (5.0-21.2)	0.249 $\pm$ 0.01 0.136 $\pm$ 0.005	106 131
3	43	15	Ventr. { Sin. (15) 12.7 $\pm$ 1.83 (9.3-14.9)	(32) 42.0 $\pm$ 2.79 (37.0-44.6) 37.4 $\pm$ 3.90 (32.6-41.7)	30.1 $\pm$ 1.34 (2.90-3.22) 17.7 $\pm$ 3.50 (16.0-24.1)	0.177 $\pm$ 0.006 0.194 $\pm$ 0.003	151 152
4	46	16	Ventr. { Sin. (21) 37.3 $\pm$ 7.61 (26.8-48.4)	(5) 33.6 $\pm$ 1.03 (32.5-34.4) 26.7 $\pm$ 2.48 (23.7-29.2)	11.2 $\pm$ 0.53 (10.9-12.1) 7.2 $\pm$ 0.32 (7.1- 7.6)	0.166 $\pm$ 0.01 0.098 $\pm$ 0.003	182 157
5	49	19	Ventr. { Sin. (10) 4.5 $\pm$ 0.59 (3.7-5.6)	(12) 27.3 $\pm$ 3.62 (22.5-31.6) 33.7 $\pm$ 8.54	9.2 $\pm$ 0.75 (9.0-10.5) 14.1 $\pm$ 6.79	0.066 $\pm$ 0.003 0.121 $\pm$ 0.008	104 139
6	67	30	Ventr. { Sin. (146) 8.05 $\pm$ 2.46	(105) 37.1 $\pm$ 7.62	18.8 $\pm$ 8.71	0.161 $\pm$ 0.012	138

The upper record of the Figure shows trans-membrane potentials recorded from the sinus venosus of the heart tube: there is a gradual decrease of membrane potential preceding the upstroke of each action potential. In fibres adjacent to the sinus region, these prepotentials were smaller and their slope was more constant.

The bottom record shows trans-membrane potentials of the same embryo, from a region which, in the future, would become ventricle: there is no prepotential. On a few occasions, the records showed waves of depolarization which were small in amplitude (1-3 mV) and never gave rise to action potentials. The two different types of trans-membrane potentials shown in the Figure were also obtained from embryos as young as 37 h.

The quantitative data are summarized in the Table. Values are separately tabulated for sinus and for ventricle. With respect to resting potentials and overshoots, they are not statistically different. The large scatter and relatively low means, particularly for the resting potential, are thought to be connected with the small dimensions of the embryonic heart cells. The average diameter was μ 4.8 $\pm$ 0.76 in cardiac tissue taken from a 37 h embryo.

The present experiments made it clear that there is a differentiation at an early stage of development: cells of the sinus region show prepotentials resembling those of adult pacemaker⁵⁻⁷; cells of the cardiac tube (future ventricles) show an electrical behaviour similar to that of adult ventricles⁶. From the findings of FÄNGE *et al.*² and the present results, it also follows that prepotentials appear (or re-appear) if cells of the heart tube are cultured *in vitro*.

E. MEDA and A. FERRONI

Istituto di Fisiologia umana della Università di Torino (Italia), July 28, 1959.

Zusammenfassung

Von embryonalen Hühnerherzen wurden *in vivo* Membranpotentiale verschiedenen Regimes registriert. Bereits auf sehr frühen Entwicklungsstadien (37 h) wird eine Differenzierung in Schrittmachergasern und spätere Ventrikelfasern gefunden.

⁵ S. WEIDMANN, J. Physiol. 115, 227 (1951).
⁶ S. WEIDMANN, Elektrophysiologie der Herzmuskel faser (H. Huber, Bern 1956).
⁷ O. HUTTER and W. TRAUTWEIN, J. gen. Physiol. 39, 715 (1956).

Die Wirkung von Harmalin auf die Konzentration von Noradrenalin und Adrenalin im Herzen

Harmalaalkaloide gehören zu den wirksamsten Hemmstoffen der Monoaminoxidase *in vitro*¹. Mit Harmalin wurden auch *in vivo* Effekte am Zentralnervensystem beobachtet, die offenbar in der Hemmung der Monoaminoxidase ihre Ursache haben². Dass auch rein periphere Wirkungen mit einem dem Harmalin nahe verwandten Stoff auftreten können, zeigten Versuche von SCRIBANE und HUTCHEON: Harmanmethosulfat führt,

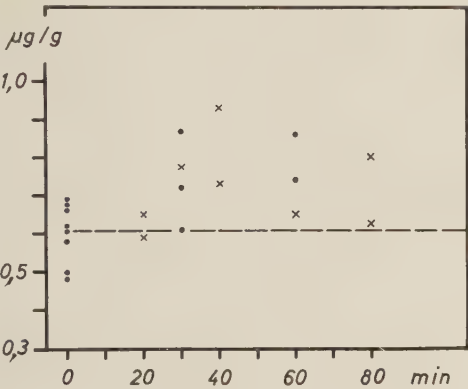
¹ K. FRETER, H. WEISSBACH, B. REDFIELD, S. UDENFRIEND und B. WITKOP, J. Amer. chem. Soc. 80, 983 (1958).
² S. UDENFRIEND und H. WEISSBACH, Proc. Soc. exp. Biol. Med. N. Y. 97, 748 (1958). - A. PLETSCHER und H. BESENDORF, Exper. 51, 25 (1959).

Tabelle

	Zeit nach Gabe von Harmalin	n	Noradrenalin	Statistische Differenz	Adrenalin	Statistische Differenz
Herzkammern	Kontrollen	8	0,60 ± 0,03	—	0,035 ± 0,005	—
	20–80 min.	13	0,74 ± 0,03	P < 0,01	0,054 ± 0,004	P < 0,02
Hirnstamm	Kontrollen	9	0,39 ± 0,02	—	nicht bestimmt	
	30 min	5	0,41 ± 0,04	P > 0,2	nicht bestimmt	
	60 min	7	0,51 ± 0,02	P < 0,001	nicht bestimmt	

Die Wirkung von Hamalin auf die Katecholamine von Herz und Hirnstamm der Ratte. n = Anzahl der Bestimmungen. Mittelwerte der Konzentration von Noradrenalin und Adrenalin in µg/g Frischgewicht ± s_x. Statistische Differenz gegenüber den Kontrollen mit dem t-Test bestimmt.

besonders in Kombination mit Adrenalin, bei Hund und Katze zu Kammerflimmern, und isolierte Papillarmuskeln werden zur Automatie angeregt³. Dies weist auf eine mögliche Beeinflussung des Katecholamin-Stoffwechsels des Herzens hin.



Zeitlicher Verlauf der Harmalinwirkung. Ordinate: Konzentration von Noradrenalin im Herzen in µg/g. Abszisse: Zeit in Minuten nach subkutaner (x) und intraperitonealer (·) Gabe von Harmalin. Jeder Wert entspricht einem Rattenherz

In den folgenden Experimenten wurde die Wirkung von Harmalin auf den Gehalt des Herzens an Noradrenalin und Adrenalin untersucht. Aus Kontrollgründen erfolgte auch die Messung der Noradrenalinkonzentration des Hirnstamms. Ratten wurde Harmalinhydrochlorid (Merck, Darmstadt) in Dosen von 5 mg/kg intraperitoneal oder 10 mg/kg subkutan verabreicht. Bei den Tieren trat innerhalb weniger Minuten ein Tremor auf, der 1–2 h anhielt und besonders ausgeprägt bei der höheren Dosis war. Die Ratten wurden in Äthernarkose entblutet und die Konzentration der Katecholamine nach der Methode von Vogt⁴ bestimmt, die für die Anwendung auf Herzgewebe bestimmte Modifikationen erfuhr⁵. Die Tabelle zeigt, dass die Konzentration der Katecholamine im Herzen durch Harmalin signifikant gesteigert wird. Auch im Hirnstamm tritt eine Erhöhung der NoradrenalinKonzentration 60 min nach der Gabe von Harmalin auf; das Ausmass der Steigerung ist aber geringer als das Ausmass der Konzentrationserhöhung von 5-Hydroxytryptamin nach HAR-

MALIN⁶. Das Maximum der Harmalinwirkung wird, wie die Abbildung zeigt, im Herz in etwa 40 min erreicht. Unterschiede in der Wirkung auf die Konzentration der Katecholamine ergaben sich zwischen den beiden benutzten Dosen nicht.

Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

E. MUSCHOLL

Pharmakologisches Institut der Universität Mainz,
17. Juni 1959.

Summary

A single injection of harmaline increases the noradrenaline and adrenaline concentration in the rat heart. The maximum effect is obtained after about 40 min.

⁶ S. UDENFRIEND und H. WEISSBACH, Proc. exp. Biol. Med., N. Y. 97, 748 (1958).

Die Pterine in der Haut und in den Augen von *Salamandra salamandra* nach Aufzucht auf verschiedenfarbigem Untergrund

Bei der Anpassung der Färbung poikilothermer Vertebraten an den Untergrund unterscheiden wir zwei Formen: den schnell erfolgenden «physiologischen» Farbwechsel, der auf einer Expansion bzw. Zusammenballung des Chromatophoren-Inhaltes beruht, und den «morphologischen» Farbwechsel, der nach langdauerndem Aufenthalt auf die Färbung des Untergrundes mit quantitativen Verschiebungen in der Chromatophorenzahl bzw. der Farbstoffmenge antwortet (vgl. ODIORNE¹).

V. FRISCH² hat die Vergrößerung der gelben Flecken bzw. der schwarzen Areale bei Feuersalamandern beschrieben, die durch Aufzucht der Larven auf gelbem bzw. schwarzem Untergrund hervorgerufen wurde. Neuerdings hat sich gezeigt, dass in den schwefelgelben Zellen des Coriums direkt unter der Epidermis sowie im retinalen Pigmentepithel der poikilothermen Vertebraten Pterine enthalten sind (GÜNDER³, ZIEGLER-GÜNDER^{4,5}).

³ A. SRIABINE und D. E. HUTCHEON, J. Pharmacol. 118, 239 (1956).

⁴ M. VOGT, J. Physiol. 123, 451 (1954).

⁵ E. MUSCHOLL, Arch. exp. Path. Pharmak., im Druck.

¹ J. M. ODIORNE, Spec. Publ. New York Acad. Sci. 4, 288 (1948).

² K. v. FRISCH, Biol. Zbl. 40, 390 (1920).

³ I. GÜNDER, Z. vgl. Physiol. 36, 78 (1954).

⁴ I. ZIEGLER-GÜNDER, Z. vgl. Physiol. 39, 163 (1956).

⁵ I. ZIEGLER-GÜNDER, Verh. dtsch. zool. Ges. 1956, 97.

Tabelle

	Isoxanthopterin und Derivate				Gelbes Pterin				Photolabiles Pterin			
	Gelb-tiere	Schwarz-tiere	n	P	Gelb-tiere	Schwarz-tiere	n	P	Gelb-tiere	Schwarz-tiere	n	P
Rumpf	626	468	39	0,0002	340	247	39	0,0002	290	210	39	0,0002
Schwanz	339	332	39	>0,05	303	298	39	>0,05	211	192	39	>0,05
Auge	—*	—*	—*	—*	213	156	9	0,0002	40	22	9	0,0002

* Substanzmenge für eine vergleichende Bestimmung zu klein

Es handelt sich dabei um Isoxanthopterin, ein dem Isoxanthopterin nahestehendes Pterin, 2-Amino-4-oxyp-
terin, ein gelbes sowie ein stark photolabiles Pterin (vgl.
ZIEGLER-GÜNDER⁶). Letzteres dürfte wie bei *Drosophila*
eine Tetrahydro-Verbindung sein, jedoch ist die Konsti-
tution sowohl des gelben als auch des photolabilen Pterins
noch nicht geklärt.

Es erhob sich nun die Frage, ob der morphologische
Farbwechsel des Feuersalamanders von einer quantita-
tiven Verschiebung in der Menge der Pterine begleitet ist.

Dazu wurden je 17 junge Larven dem Uterus eines
Muttertieres entnommen und wechselweise in zwei Glas-
becken gesetzt, die mit Plaka-Farbe allseitig schwarz bzw.
gelb gestrichen und mit Zaponlack überzogen waren.
Nach 6 Wochen (Wassertemperatur 14–16° C, Fütterung
mit lebenden Tubifex) standen die Tiere kurz vor der
Metamorphose.

Die auf gelbem Untergrund gezogenen Tiere (=Gelbtiere)
zeigten wesentlich grössere gelbe Areale als die Schwarz-
tiere: die Melaninbezirke zwischen den einzelnen gelben
Flecken waren im Vergleich zu den Schwarztieren teil-
weise verschwunden und mit gelbem Pigment erfüllt, so
dass bei den meisten Tieren neben zwei durchgehenden
gelben Längsstreifen ausgedehnte Gelbareale an den
Flanken entstanden waren.

Zur quantitativen Bestimmung der Pterine wurde die
Haut mit der anhängenden Muskulatur abpräpariert, in
zwei Teile geteilt (1. Rumpf: zwischen Vorderbeinen und
Hinterbeinen; 2. Schwanzteil), sowie die Augen ausge-
nommen und alles im Dunkeln bei 60° C getrocknet. Die
fein zerriebenen Proben wurden mehrmals mit *n*/10 HCl
bei 50° C im Dunkeln 5–6 h lang extrahiert und die ver-
einigten Extrakte im Vakuum über KOH zur Trockne
eingedampft. Nach Auflösen in einer konstanten Menge
n/10 NH₄OH und Chromatographie gleichgrosser Proben
mit Butanol/Eisessig/Wasser (4:1:5) ergaben sich 3 Pte-
rinzonen: eine violettblau fluoreszierende (vorwiegend
Isoxanthopterin und ihm nahestehende Pterinezone), das
gelbgefärbte und gelb fluoreszierende sowie das stark
photolabile Pterin⁷.

Die quantitative Ausmessung erfolgte mit einem Fluor-
ometer zur Auswertung von Papierchromatogrammen
(vgl. KÜHN⁸) und ergab folgende Resultate (Tabelle).

Wir sehen, dass in der *Rumpffregion* bei den Gelbtieren
sämtliche Pterine – nicht nur das als gelblicher Farbstoff
sichtbare – um etwa 35% zunehmen. Die Differenzen sind
gut gesichert.

⁶ I. ZIEGLER-GÜNDER, Z. Naturf. 11b, 493 (1956).

⁷ Durch die Behandlung mit warmer *n*/10 HCl sind die Pterin-
komponenten qualitativ gegenüber sehr milden Extraktionsbedin-
gungen (z. B. *n*/50 NH₄OH) teilweise verändert. Eine quantitative
Extraktion wäre aber in diesem Falle nicht möglich. Das gelbe
und das photolabile Pterin sind jedoch unverändert erhalten.

⁸ A. KÜHN: Naturwiss. 42, 529 (1955).

Auch das gelbe Pterin im *retinalen Pigmentepithel* steigt
um ungefähr den gleichen Betrag (Differenz gut ge-
sichert), während das photolabile sogar um 82% gegen-
über den Schwarztieren vermehrt ist.

Der «morphologische» Farbwechsel ist beim Feuer-
salamander somit von einer quantitativen Verschiebung
in der Pterinmenge begleitet. Weitere Untersuchungen
müssten zeigen, ob auch andere Pigmente – zum Beispiel
das in der Epidermis der gelben Areale gelegene Caroti-
noid – gleichsinnige Veränderungen aufweisen.

Bemerkenswert ist die starke Zunahme des *photolabilen*
Pterins in *retinalen Pigmentepithel* der Gelbtiere im
Gegensatz zur Haut, wo das Verhältnis gelbes Pterin/
photolabiles Pterin konstant bleibt. Die relativ stärkere
Zunahme des photolabilen Pterins kann einerseits durch
eine stetig höhere Zuwachsrate, andererseits durch ein
rascheres Einsetzen der Synthese im Vergleich zu den
anderen Pterinen bzw. im Vergleich zur Haut bedingt
sein. Da die Anpassung an den Untergrund durch das
Auge vermittelt wird (vgl. ODIORNE¹), könnte eine nähere
Analyse vielleicht Einblicke in die noch völlig unbe-
kannten kausalen Zusammenhänge zwischen Bodenfarbe
und Färbung des Tieres bringen.

IRMGARD ZIEGLER-GÜNDER*

Zoologisches Institut der Universität München, 5. Mai
1959.

Summary

Concomitantly with the morphological colour-change,
larvae of *Salamandra salamandra* show a quantitative
change in their pteridines. After being reared on a yellow
background, all pteridines of the skin (isoxanthopterin
and its derivatives, yellow pteridine, photosensitive
pteridine) increase by about 1/3 as compared with the
animals on a black background. In the retinal pigment-
layer especially, the photosensitive pteridine is augmented.

* Jetzige Anschrift: Botanisches Institut der Technischen Hoch-
schule Darmstadt.

A Suggested Mechanism for the Action of Choline
Esters on Animal Organs, inferred from a Study
of the Effect of Choline-, β-methylcholine-, and
Thiocholine-esters

In previous works^{1,2}, we suggested that the rate-
determining step in the action of a choline (Ch) ester on

¹ M. WURZEL, XXth Int. Congr. Physiol. Brussels 1956, Abstracts
of Communications, p. 980.

² M. WURZEL and N. SAPEIKA, S. A. J. lab. clin. Med. 4, 229
(1958).

Table I
Muscarinic effects and enzymatic hydrolysis / Choline-moiety kept constant, acyl changing

I. Hydrolysis by Enzyme*		Ac	Prop	But	Benz
		True ChE			
Choline		1	1-3.7	100-200	∞
β-methylcholine		1	1.9	200<	∞
Thiocholine		1	1.2	125	∞
II. Potency on Organs		Muscarinic responses			
Choline	guinea-pig intestine . . .	1	19	510	2,600
	cat blood pressure fall . .	1	37	2,900	110,000
β-methylcholine	guinea-pig intestine . . .	1	24	4,500	500,000 <
	cat blood pressure fall . .	1	39	820	82,000
Thiocholine	cat blood pressure fall (atropine sensitive) . . .	slightly active	inactive	inactive	inactive?

The figures for the enzyme are relative rates of hydrolysis obtained by dividing the rate of hydrolysis of the acetyl ester by the rates of hydrolysis of each of the other esters, thus the rate of hydrolysis of the acetyl ester is taken as unity.

The figures for the organs are relative equipotent molar concentrations obtained by dividing the equipotent molar concentration of each ester by that of the acetyl ester. Thus higher figures represent lower rates of hydrolysis and lower potencies respectively.

The figures for SCh ester hydrolysis were computed from KOELLE⁶, except that for PropSCh.

a living organ might be its rate of hydrolysis by one of the cholinesterases (ChE). The experimental evidence for this was the observation that a series of choline esters of carboxylic acids, when applied to different animal organs, showed an order of potency parallel to the order of their rates of enzymatic hydrolysis, both with true ChE (in the case of ‘muscarinic-’) and with pseudo ChE (in the case of ‘nicotinic-’ effects). It was also shown^{3,4} that when equipotent doses of different choline esters, i.e. concentrations causing half the maximum contraction of a muscle, are added to an identical concentration of true- or pseudo-ChE, they yield the same amount of reaction products (choline + carboxylic acid) per unit of time. For example: in the case of smooth muscle contraction, 500 molar concentration units of butyrylcholine (ButCh) are equipotent with 1 of acetylcholine (ACh); furthermore, 100–200 such units of ButCh give the same amount of end-products per minute as 1 unit of ACh, when hydrolyzed by an identical concentration of true ChE.

Carbaminoylcholine (CarbCh) is biologically as potent as ACh, but is not hydrolyzed by ChE-s. Since CarbCh is a carbamic acid ester, it differs from ACh only in having the CH₃-group of acetic acid replaced by a NH₂-group. It might therefore be thought that the receptor, i.e. the sensitive site on the organ, on which a compound added from outside acts, would be the same for both ACh and CarbCh. It might then be argued that as hydrolysis plays no part in the biological action of CarbCh, neither does it do so in the case of ACh.

According to WILSON and NACHMANSOHN⁵, in the biological activity of carboxylic choline esters, ‘hydrolytic activity is not a prerequisite for receptor activity’; however they also add that ‘although there is no proof that such is the case, neither is there proof to the contrary’.

In our present investigation, the correlation between hydrolysis by ChE and biological potency was further studied with two additional series: β-methylcholine-(MeCh) and thiocholine-(SCh)esters. This choice was made since it is well known⁷ that acetyl-β-methylcholine (AcMeCh) has predominantly muscarinic properties and weak nicotinic ones, and acetyl-thiocholine (ASCh) has strong nicotinic but barely discernible muscarinic effects, whereas ACh has both muscarinic and nicotinic properties⁷.

Results. The enzymatic and biological effects of the above three sets of compounds are compared in Tables I and II. The choline- or choline-derivative moiety was kept constant, while the acyl part was in turn acetyl- (Ac), propionyl- (Prop), butyryl- (But), and benzoyl- (Benz). Despite the fact that the comparison was made between a homogenous enzyme solution on the one hand and highly organized biological structures on the other, the figures are on the whole in fairly close agreement. It may be concluded, therefore, that the orders of decreasing rates of enzymatic hydrolysis and of decreasing biological potency are parallel. Thus Table I and II strongly support the view that the rate-determining, perhaps the first, step in the biological action of these choline esters is their hydrolysis by one of the ChE-s.

In Tables III and IV, enzymatic hydrolysis rates and biological activity are compared, the acyl moiety being in all cases acetyl, and the alcohol moiety being in turn Ch, MeCh, and SCh. In this series no striking correlation can be detected between biological potency and rates of enzymatic hydrolysis: AcMeCh has a stronger muscarinic effect than could be foreseen from hydrolysis; ASCh is very weakly muscarinic, inspite of being a very good substrate for the ChE-s; the nicotinic potency of ASCh is high but perhaps less than would be expected from the rate of hydrolysis.

³ M. WURZEL, Bull. Res. Council Israel 5A, 303 (1956).

⁴ M. WURZEL, submitted for publication A (1959).

⁵ I. B. WILSON and D. NACHMANSOHN, in *Ion Transport Across Membranes* (by H. T. Clarke, Ed., Academic Press, New York 1954), p. 62.

⁶ G. B. KOELLE, J. Pharmacol. exper. Therap. 100, 160 (1950).

⁷ D. BOVET and F. BOVET-NITTI, *Médicaments du système nerveux végétatif* (Ed. S. Karger, Bâle 1948), p. 375 and chapters VIII, IX, X.

Table II
Nicotinic effects and enzymatic hydrolysis / Choline-moiety kept constant, acyl changes

I. Hydrolysis by Enzyme		Ac	Prop	But	Benz
		Pseudo ChE			
Choline		1	0.5	0.4	3.4
β -methylcholine		1			
Thiocholine		1	0.6	0.7	11
II. Potency on Organs		Nicotinic responses			
Choline	frog rectus muscle	1	0.7	0.3	21
	cat-atropinized blood pressure rise	1	0.8	0.5	2.3
β -methylcholine	frog rectus muscle	1	2.1	2.1	57
	cat-atropinized blood pressure rise	inactive (complex responses)	inactive responses	active	active
Thiocholine	frog rectus muscle	1	3	2.3	<100
	cat blood pressure rise . .	1	1.1	0.6	2
	guinea-pig intestine, nicotinic contraction ⁹ . . .	1	2	2.5	6

The figures: as described for Table I.

⁹ M. WURZEL, submitted for publication B (1959).

The above data suggest the existence of two stages in the action of choline esters on animal organs: one dependent on ChE hydrolytic activity, and the second independent of ChE.

Stage A would consist of hydrolysis by one of the two ChE-s and would produce Ch, MeCh, or SCh as reaction products necessary for the next stage.

Stage B. The choline-derivative obtained by hydrolysis in stage A would react with the hypothetic receptor-component B, it too having its own substrate specificity: the muscarinic receptor is more sensitive to MeCh than to Ch, but is insensitive to SCh; the nicotinic receptor is sensitive to Ch and SCh, as well as to MeCh, but this latter is only available when high concentrations of AcMeCh are added, its hydrolysis by pseudo ChE of the 'nicotinic' organ being slower. While there is no doubt that the receptor-component of stage B has an easily definable substrate specificity, its nature is still a subject for speculation.

The apparent discrepancy, between the theory that ester hydrolysis is essential for biological activity and the fact that CarbCh is a stable but nevertheless potent compound, could be accounted for by assuming that CarbCh would by-pass stage A, directly entering the reaction at stage B. Its β -MeCh derivative was shown to have musca-

rinic, but no nicotinic effect, in analogy to the carboxylic ester of MeCh⁶. Thus, sensitivity to the β -methyl grouping seems to be characteristic of the receptor component of stage B, which is acted upon both by unhydrolyzed carbaminoyl- β -methylcholine and the hydrolyzable carboxylic acid esters of β -methylcholine.

As evidence of hydrolytic activity not being a prerequisite for receptor activity, WILSON and NACHMANSON⁵ compared the behaviour of ButCh, ACh, and dimethylaminoethylacetate (tertACh) towards the enzyme-true ChE, with their action on the receptor. The above authors state that: (1) ButCh and ACh are both good stimulators of the receptor of the frog rectus muscle, but ButCh is a very poor true ChE enzyme substrate. They further claim (2) tertACh is a good substrate for the enzyme, but acts poorly on the receptor. On the other hand, our arguments, as deduced from Table I-IV, are:

Table IV
Nicotinic effects and enzymatic hydrolysis
Choline-moiety changes, acyl constantly acetyl

I. Hydrolysis by Enzyme	ACh	A β MeCh	ASCh
	Pseudo ChE		
	1	72	0.23
II. Potency on Organs	Nicotinic responses		
Frog rectus muscle	1	180	20-40
Cat-atropinized blood pressure rise	1	certainly high	0.2
Guinea-pig intestine, nicotinic contraction	?	?	very potent, but ratio to ACh is not definable

Table III
Muscarinic effects and enzymatic hydrolysis
Choline-moiety changes, acyl constantly acetyl

I. Hydrolysis by Enzyme	ACh	A β MeCh	ASCh
	True ChE		
	1	5.7	0.7
II. Potency on Organs	Muscarinic responses		
Guinea-pig intestine . .	1	1.3	20 $\ll$
Cat blood pressure fall .	1	1.4	100,000 <

The figures: as in Table I, except ACh being always taken as unity.

The figures: as in Table I, except ACh being always taken as unity.

(1) the enzyme acting on the receptor of the frog rectus muscle is pseudo ChE (see Table II and ⁸), therefore ButCh is a good substrate of the enzyme pseudo ChE and also a good receptor-stimulator;

(2) tertACh has little or no biological effect on the receptor, because its hydrolysis in stage A produces dimethylaminoethanol (tertCh) which probably has a low affinity for the stage B receptor-component (compare with Table III, where the acyl moiety is kept constant, while the choline moiety changes). Thus tertACh's behaviour is analogous to that of ASCh towards the muscarinic receptor, ASCh being a very good substrate for ChE-s, even though it has no affinity for the muscarinic receptor.

M. WURZEL

The Hebrew University, Hadassah Medical School,
Department of Physiology, Jerusalem (Israel), June 5,
1959.

Résumé

Le mécanisme d'action des esters carboxyliques de la choline commence, probablement, par une hydrolyse enzymatique provoquée par une des cholinestérases. Les produits de l'hydrolyse réagissent avec un facteur inconnu, dont la spécificité est cependant définissable par la structure du résidu cholinique.

⁸ J. DECHAVASSINE, Exper. 12, 434 (1956).

Observations

on Spontaneous Hemolysis in Shed Blood

The existence of a relation between the content of reduced glutathione of erythrocytes and their rate of spontaneous hemolysis has been suggested by various workers (LEMBERG and LEGGE¹).

FEGLER² concluded from a study of horse blood that the rate of hemolysis bears a certain relation to the extent of oxidation of the reduced glutathione. KEILIN and HARTREE³ found that the treatment of horse erythrocytes with sodium nitrite, though it produced complete oxidation of the reduced glutathione and converted the hemoglobin to methemoglobin, did not result in hemolysis of the erythrocytes until after 12 hours of storage in the cold. Since these two views appear to be mutually exclusive, a study of the relationship between the rate of hemolysis of nitrite treated and untreated erythrocytes and their glutathione content was attempted.

Blood samples from 10 Bali oxen (*Bos banteng*), 3 monkeys (*Macaca cynomolgus*), and 1 horse were collected in flasks containing potassium oxalate crystals, while those from 4 normal human subjects were collected in flasks containing heparin. The erythrocytes were washed with a 1% NaCl aqueous solution, 3–4 times with centrifuging. Aliquots were treated with equal volumes of an isotonic solution of NaNO₂ (1.06% in water) for 1–2 min and the nitrite was removed using the saline, washing 3–4 times, and centrifuging. The treated and untreated erythrocytes were washed twice with isotonic saline which

contained: Streptomycin, 1 mg/ml, and 1000 units of penicillin, and then measured volumes of these cells were suspended, separately, in equal amounts of the isotonic saline. These cells were contained in centrifuge tubes, plugged with cotton wool and kept in a water bath at 37°C. The total hemoglobin and hemoglobin in the supernatant fluid were measured by a modification of the method for plasma hemoglobin (DACIE⁴). Reduced glutathione was measured by the nitroprusside colour reaction of THOMPSON and WATSON⁵. The type of hemoglobin contained in the erythrocytes was determined by a paper electrophoretic technique (VELLA⁶). Determinations of reduced glutathione in the erythrocyte suspension and hemoglobin in the supernatant fluid were made at intervals of 0, 3, 6, 10, and 24 h.

The treatment of the erythrocytes with NaNO₂ oxidised the glutathione completely. In the untreated erythrocytes the content of reduced glutathione fell gradually to 8–40% (average: 20%) in ox, to 15–50% (average: 36%) in monkey, to 53% in the horse, and to 10–16% (average: 26%) in the human samples. The amount of hemoglobin in the supernatant fluid after 24 h at 37°C amounted to between 0.5 and 2.5% of the total hemoglobin at zero time. No significant differences were detected in the rates of spontaneous hemolysis in the nitrite treated and untreated erythrocytes from the same blood sample, though the rate of hemolysis appeared to be specific for each blood sample. The type of hemoglobin present (A, B, C, AB, or AC) in the samples from oxen did not appear to be related to the rate of spontaneous oxidation of the glutathione in the untreated cells or the rate of hemolysis of either the treated or the untreated samples. The hemoglobin in the horse, the monkey, and the human samples was electrophoretically homogeneous and normal in type for each species.

Spontaneous oxidation of reduced glutathione of shed blood is only one of many chemical changes taking place in red blood cells kept under conditions similar to those employed in these experiments. Loss of bicarbonate, conversion of glucose to lactic acid, loss of inorganic phosphate from breakdown of ester phosphate in the cells, loss of K⁺ and gain in Na⁺ (VARLEY⁷), loss of 'labile' iron and bilirubin (BARKAN and WALKER⁸) and of pyruvate (LONG⁹) are well known occurrences in the human shed blood.

The treatment of erythrocytes with NaNO₂ produces complete oxidation of reduced glutathione and converts oxyhemoglobin to methemoglobin but does not affect the catalase and the carbonic anhydrase activity (KEILIN and HARTREE³); it also denatures the globin of hemoglobin producing 'nitrite cat-hemoglobin' (BARNARD¹⁰). Methemoglobin formation *per se* does not appear to damage the erythrocytes (LEMBERG and LEGGE¹).

From the present experiments, it appears that the rate of spontaneous hemolysis of erythrocytes from several species is independent of their content of reduced glutathione and is not affected adversely by the marked changes produced by treatment with nitrite.

Acknowledgment. I am grateful to Dr. LEE LIANG HIN of the Department of Bacteriology, Faculty of Medicine, University of

⁴ J. V. DACIE, *Practical Haematology* (J. & A. Churchill Ltd., London 1956), p. 139.

⁵ R. H. S. THOMPSON and D. WATSON, *J. clin. Pathol.* 5, 25 (1952).

⁶ F. VELLA, *Nature* 181, 564 (1958).

⁷ H. VARLEY, *Practical Clinical Biochemistry* (William Heinmann Medical Books, Ltd., London 1958), p. 7.

⁸ G. BARKAN and B. S. WALKER, *J. biol. Chem.* 131, 447 (1939).

⁹ C. LONG, *Biochem. J.* 38, 447 (1944).

¹⁰ R. D. BARNARD, *J. biol. Chem.* 120, 177 (1937).

¹ R. LEMBERG and J. W. LEGGE, *Hematin Compounds and Bile Pigments* (Interscience Publishers, Inc., New York 1949), p. 517 and 523.

² G. FEGLER, *Nature* 170, 624 (1952).

³ D. KEILIN and E. G. HARTREE, *Nature* 157, 210 (1946).

Malaya in Singapore for making available blood samples from *Macaca cynomolgus*, to Mr. J. T. FORBES of the Singapore Turf Club for providing the horse blood, and to Mr. STEPHEN PANG for his technical assistance.

F. VELLA

Department of Biochemistry, Faculty of Medicine, University of Malaya, Singapore, June 25, 1959.

Résumé

Le traitement des érythrocytes de bœuf (*Bos banteng*), de singe (*Macaca cynomolgus*), de cheval et de sujets humains avec du nitrite de sodium n'a pas d'influence sur la rapidité de l'hémolyse spontanée.

Bicarbonate Excretion after Prolonged Exposure to Carbon Dioxide in the Normal Dog

SULLIVAN and DORMAN¹ have shown that exposure of dogs to an atmosphere containing approximately 10% CO₂ during periods extending from 2 to 77 days, markedly increased the tubular reabsorption of bicarbonate. This increment was much more important than in experiments where arterial $p\text{CO}_2$ was acutely raised to similar levels. No satisfactory explanation was given to this phenomenon.

Previous work from this laboratory^{2,3} demonstrated that hypochloremia increases tubular bicarbonate reabsorption. On the other hand, hypochloremia is a common component of the plasma electrolyte pattern of chronic respiratory acidosis in the human⁴. These facts seemed to afford a clue to the understanding of the process which increases the reabsorption of bicarbonate by the renal tubules during prolonged exposure to CO₂. The data to be presented demonstrate that the further increase in bicarbonate reabsorption observed during chronic respiratory acidosis as compared to acute respiratory acidosis, is intimately linked to the hypochloremia which characterises the chronic condition.

Methods. 9 female dogs, weighing from 11 to 29 kg, were used in 3 types of experiments.

(1) **Controls** (5 experiments, 54 clearance periods): intact dogs were infused with a solution of 1.2% NaHCO₃ in 0.1% creatinine at rates ranging from 8 to 10 ml/min, after priming doses of 6 g NaHCO₃ and 1 g creatinine. Plasma bicarbonate concentration was increased in a stepwise manner by 3 successive priming doses of 3 to 9 g bicarbonate, according to the size of the animal, injected intravenously at 35–45 min intervals. Between these injections, the sustaining infusion was pursued at constant rate, and arterial blood and urine were collected anaerobically.

The usual clearance method was used throughout with urine collection periods ranging from 8 to 12 min. From 10 to 15 clearance periods were obtained for each experiment.

(2) **Acute respiratory acidosis** (5 experiments, 60 clearance periods): the same schedule was used as in the control experiments, except that the animals were breathing 8–12% CO₂ in oxygen under very light Pentothal anesthesia during the whole procedure. Plasma bicarbonate concentration was similarly raised by 3 successive priming doses of 3 to 9 g NaHCO₃.

(3) **Chronic respiratory acidosis** (9 experiments): the dogs were maintained during 4 to 6 days in an oxygen tent containing approximately 50% oxygen and from 7 to 10% CO₂. These animals were divided into 2 groups:

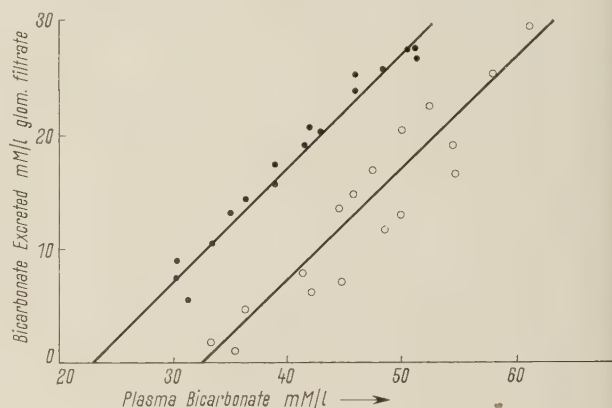


Fig. 1

● Controls	{ mean PCI = 99 mEq/l (89–110)
	{ mean $p\text{CO}_2$ = 46 mmHg (42–51)
○ Chronic CO ₂ -exposure	{ mean PCI = 81 mEq/l (76–90)
	{ mean $p\text{CO}_2$ = 54 mmHg (39–68)

Fig. 1.—Bicarbonate excretion rate in intact control dogs (black circles) and in dogs previously submitted to 4–6 days CO₂ exposure but breathing room air while infused with bicarbonate solution (open circles). Each symbol represents the mean of 3 successive urine collection periods. The mean values of $p\text{CO}_2$ and plasma chloride concentration (PCI) for each group are presented.

(a) *in the first group* (4 experiments, 54 clearance periods) after their removal from the tent, the dogs were submitted to the same procedure as in the controls,

(b) *in the second group* (5 experiments, 66 clearance periods), 1 to 3 min after their removal from the tent, the animals were lightly anesthetised with Pentothal and submitted to the same procedure as in the acute respiratory acidosis experiments.

Results. (1) **Controls** (see Fig. 1): In Figure 1, the excretion rate of bicarbonate in the intact dogs has been expressed in mM/l of glomerular filtrate (mM/l GF) and the mean values of this ratio, calculated from 3 successive clearance periods, plotted with corresponding plasma bicarbonate concentration mean values. The linear correlation between bicarbonate excretion rate and plasma bicarbonate concentration is similar to that observed by PITTS and LOTSPEICH⁵ in the same type of experiments. The relationship meets the abscissae at a plasma concentration value of 22.5 mM/l which constitutes the theoretical threshold for bicarbonate. The slope of the curve is given by the ratio (bicarbonate excretion rate, mM/l GF)/plasma bicarbonate concentration mM/l-22.5). This ratio has a mean value of 0.970 for the 5 experiments instead of a theoretical value of 1.00. It should be noted that plasma chloride concentration has a mean value of 99 mEq/l (range 89–110) during these control experiments.

¹ W. J. SULLIVAN and P. J. DORMAN, J. clin. Invest. 34, 268 (1955).

² CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. 14, 417 (1958).

³ CH. TOUSSAINT, M. TELERMAN, and P. VEREERSTRAETEN, Exper. 15, 232 (1959).

⁴ J. R. ELKINTON and T. S. DANOWSKI, *The Body Fluids, Basic Physiology and Practical Therapeutics* (Williams and Wilkins Co., Baltimore 1955).

⁵ R. F. PITTS and W. D. LOTSPEICH, Amer. J. Physiol. 147, 138 (1946).

(2) *Acute respiratory acidosis* (see Fig. 2): Figure 2 shows in a similar manner the correlation between bicarbonate excretion rates and plasma bicarbonate concentrations in experiments where arterial $p\text{CO}_2$ was acutely raised in otherwise intact animals. The slope of the curve (0.942) is virtually the same as in the controls but the bicarbonate threshold value has been increased to 27.5 mM/l, due to the rise of arterial $p\text{CO}_2$ mean value from 46 to 73 mm Hg. During these experiments, plasma chloride concentration has a mean value of 94 mEq/l (range 83–106).

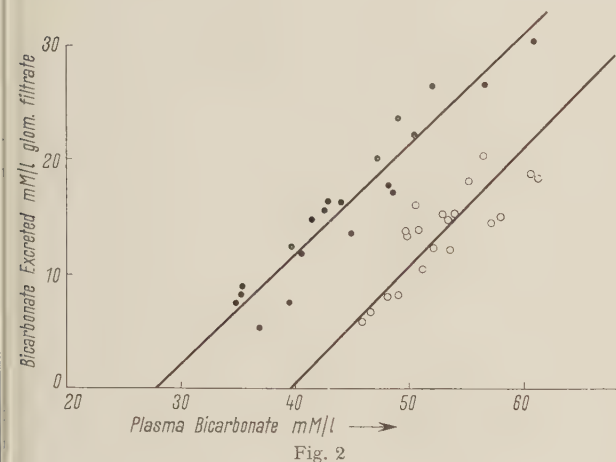


Fig. 2.—Bicarbonate excretion rate during acute respiratory acidosis (black circles) and in dogs previously submitted to 4–6 days CO_2 exposure and breathing 8–12% CO_2 while infused with bicarbonate solution (open circles). Same presentation as in Figure 1.

(3) *Chronic respiratory acidosis* (Fig. 1 and 2):

(a) *In animals breathing room air*: within a few minutes after their removal from the ' CO_2 tent', these dogs show a precipitous fall in arterial $p\text{CO}_2$ due to the high diffusibility of the gas. At this moment, plasma bicarbonate concentration is still at a high value because of the slowness of the renal adjustment mechanism to the acute decrease in arterial $p\text{CO}_2$. Figure 1 shows that the bicarbonate excretion rate-plasma concentration relationship has a slope (0.900) which is not significantly different⁶ from that observed during bicarbonate infusion in intact dogs. Indeed the data are somewhat more scattered due to the larger dispersion of the $p\text{CO}_2$ values in the chronic experiments. It is however apparent that the bicarbonate threshold value has been increased to 32.0 mM/l by the previous exposure to CO_2 . The mean value of plasma chloride concentration observed in these experiments is 79 mEq/l (range 72–90). In a first stage, chloremia is decreased from 112 (range 111–115) to 98 mEq/l (range 96–104) by the prolonged exposure to CO_2 . In a second stage, it is further depressed to the 79 mEq/l level by the bicarbonate infusion.

(b) *In animals breathing 8–12% CO_2* : it is readily apparent from Figure 2 that for a given arterial $p\text{CO}_2$ value of approximately 70 mm Hg tubular bicarbonate re-

absorption has been increased by the prolonged exposure to CO_2 in the tent: the plasma threshold value rises from 27.5 in acute respiratory acidosis to 39.7 mM/l in the chronic condition. Here again the slope of the bicarbonate excretion rate-plasma concentration relationship remains constant (1.029). As for the preceding group, plasma chloride concentration decreases simultaneously to a mean value of 81 mEq/l (range 73–92).

Discussion. Our data confirm the important increase in tubular bicarbonate reabsorption observed by SULLIVAN and DORMAN¹ after prolonged exposure of dogs to CO_2 .

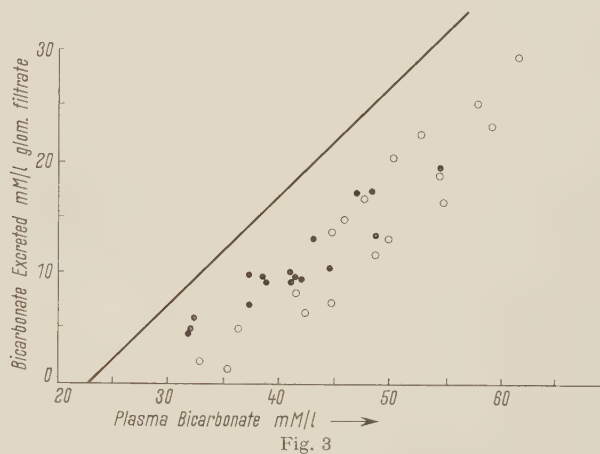


Fig. 3.—Bicarbonate excretion rate in dogs made hypochloremic by hemodialysis or peritoneal lavage (black circles) and in dogs previously submitted to 4–6 days CO_2 exposure while infused with bicarbonate solution (open circles). Same presentation as in Figure 1.

The observations made on the animals breathing room air after removal from the ' CO_2 tent' further show that bicarbonate reabsorption is also increased at normal $p\text{CO}_2$ levels.

The increment of tubular bicarbonate reabsorption could be explained by one of the following hypotheses: (1) a fall of GFR would increase the renal bicarbonate threshold value; (2) prolonged exposure to CO_2 could enhance the activity of the carbonic anhydrase system of the tubules; (3) prolonged exposure to CO_2 could lead to some potassium depletion in the tubular cells; (4) the hypochloremia following prolonged exposure to CO_2 would be of a sufficient magnitude to account for the increase in bicarbonate reabsorption.

Hypothesis 1: THOMPSON and BARRETT⁷ have shown in dogs that an acute fall in GFR is followed by an increase in bicarbonate threshold value because bicarbonate reabsorption by the tubules remains unabated despite the reduction of the bicarbonate filtered load. To rule out the possibility that a fall in GFR during prolonged exposure to CO_2 would be responsible for the increase in bicarbonate threshold, GFR was estimated by the endogenous creatinine clearance, using 24 h urine collection periods, in dogs observed in metabolic cages before and during prolonged exposure to CO_2 . In none of 6 such experiments was a drop of GFR observed. Moreover, GFR constantly rises during the acute experiments performed

⁶ Statistical calculation actually shows that there are no significant differences between the slopes of the bicarbonate excretion rate-plasma concentration relationship obtained in the 4 different types of experiments.

⁷ D. D. THOMPSON and M. J. BARRETT, Amer. J. Physiol. 176, 201.

after prolonged CO_2 exposure as it is usual to observe in dogs by the rapid infusion of any electrolyte solution⁸. It can be concluded that a decrease in GFR does not account for the increment of bicarbonate threshold values in these experiments.

Hypothesis 2: A change in the activity of the carbonic anhydrase system of the renal tubules seems improbable, since CARTER *et al.*⁹ could not find any change in carbonic anhydrase concentration in the kidney of rats submitted to prolonged exposure to CO_2 .

Hypothesis 3: Potassium depletion of the tubule cells would constitute a better explanation for the enhancement of bicarbonate reabsorption following prolonged CO_2 exposure, since the reabsorption of this ion was found to be increased during potassium depletion in the dog¹⁰ and in the human¹¹. That prolonged exposure of dogs to CO_2 does not induce negative potassium balance is readily apparent from metabolic studies conducted in 6 dogs during chronic CO_2 exposure. It should be noted, however, that the usual drop in plasma potassium concentration following NaHCO_3 infusion is always more pronounced in animals previously submitted to prolonged CO_2 exposure. However, a direct action of hypokaliemia itself on bicarbonate reabsorption seems to be ruled out by the lack of effect of insulin or bicarbonate-induced hypokaliemia on bicarbonate reabsorption¹¹.

Hypothesis 4: Hypochloremia induced by renal adjustment to chronic respiratory acidosis¹² seems to constitute the main cause of the enhancement of tubular bicarbonate reabsorption following prolonged exposure to CO_2 atmosphere. Comparison between control dogs and animals breathing room air but having previously inhaled CO_2 for 4 to 6 days (Fig. 1) shows the following points: (1) arterial $p\text{CO}_2$ has practically the same mean value in both groups (46 and 51 mm Hg, respectively); (2) plasma chloride concentration values are very different: 99 mEq/l in the controls and 79 mEq/l in the experiments involving previous exposure to CO_2 ; (3) bicarbonate threshold values vary accordingly: 22.5 mM/l in the controls and 32.0 mM/l in the second group of experiments.

Comparison between the acute respiratory acidosis experiments, and similar experiments involving previous exposure of the animals to CO_2 atmosphere for 4 to 6 days, yields identical results (Fig. 2): (1) arterial $p\text{CO}_2$ has the same mean value in both groups (73 and 76 mm Hg); (2) plasma chloride concentration values are 94 and 81 mEq/l respectively; (3) bicarbonate threshold values are 27.5 and 39.7 mM/l respectively. It can be seen that for the two groups of chronic experiments a mean decrease of some 17 mEq/l in plasma chloride concentration is accompanied by a mean increase of approximately 11 mM/l in bicarbonate threshold value for similar arterial $p\text{CO}_2$ levels.

In Figure 3, bicarbonate excretion rates at normal $p\text{CO}_2$ levels in dogs previously submitted to prolonged CO_2 exposure are compared to bicarbonate excretion rates

(51 clearance periods) at slightly higher arterial $p\text{CO}_2$ levels in 13 animals made acutely hypochloremic by hemodialysis² or peritoneal lavage³. Plasma chloride concentration is decreased to approximately the same mean value (84 and 81 mEq/l respectively) in both groups.

There is obviously no difference between the bicarbonate excretion rate-plasma concentration relationships in the two types of hypochloremia investigated. Thus, despite entirely different experimental procedures and marked difference in durations of induction, an equal drop in plasma chloride concentration is accompanied by a rise in tubular bicarbonate reabsorption of an equal magnitude.

On the other hand, an increase in plasma chloride concentration has been shown to decrease bicarbonate reabsorption in the dog, during acute respiratory acidosis¹³ or at normal $p\text{CO}_2$ levels⁵.

It is concluded that hypochloremia is the main cause, perhaps the only cause, of the supplementary increase in tubular bicarbonate reabsorption observed after prolonged exposure to CO_2 . The mode of action of the Cl ion on the bicarbonate reabsorption mechanism is unknown.

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Résumé

L'excrétion du bicarbonate a été étudiée chez des chiens ayant préalablement séjourné pendant des périodes de 4 à 6 jours dans une atmosphère contenant de 7 à 10% de CO_2 .

Ce procédé a pour effet d'accroître fortement la réabsorption tubulaire du bicarbonate par un mécanisme distinct de l'action de l'accroissement de la pression partielle du CO_2 et analogue à celui qui augmente la réabsorption du bicarbonate au cours de l'hypochlorémie aiguë réalisée par des procédés de vividialyse.

¹³ J. G. HILTON, N. E. CAPECI, G. T. KISS, O. R. KRUESI, V. V. GLAVIANO, and R. WEGRIA, *J. clin. Invest.* 35, 481 (1956).

Oxydation von Bernsteinsäure durch Leber akut urämischer Ratten¹

5. Mitteilung
über Untersuchungen der Rest-N-fractionen bei Urämie

In der akut urämischen Rattenleber sind Desaminierung und Transaminierung von DL-Alanin signifikant gesteigert². Dieser Befund ist für den urämischen Organismus in zweifacher Hinsicht bedeutungsvoll.

Aminosäuren werden entweder zu Ketosäuren desaminiert bzw. transaminiert oder zu Aminen dekarboxyliert. Bekanntlich haben Amine an Mensch und Tier pharmakologisch-toxische Wirkungen. Da bei akuter experimen-

¹ Die vorliegende Arbeit wurde mit Unterstützung des Schweizerischen Nationalfonds durchgeführt.

² R. BOSSHARDT, H. THÖLEN und F. ENDERLIN, *Exper.* 13, 497 (1957).

⁷ D. D. THOMPSON and M. J. BARRETT, *Amer. J. Physiol.* 176, 201 (1954).

⁸ L. G. WESSON, JR., W. P. ANSLOW, JR., L. G. RAISZ, A. A. BOLOMEY, and M. LADD, *Amer. J. Physiol.* 162, 677 (1950).

⁹ N. W. CARTER, D. W. SELDIN, and H. C. TENG, *J. clin. Invest.* 38, 949 (1959).

¹⁰ Unpublished data.

¹¹ K. E. ROBERTS, H. T. RANDALL, H. L. SANDERS, and M. HOOD, *J. clin. Invest.* 34, 666 (1955).

¹² H. LEVITIN, W. BRANSCOME, and F. H. EPSTEIN, *J. clin. Invest.* 37, 1667 (1958).

teller Urämie die Desaminierung von Aminosäuren vermehrt ist, darf man wohl annehmen, dass weniger Aminosäuren zu toxischen Aminen dekarboxyliert werden. In diesem Sinne wirkt die akut urämische Rattenleber entgiftend.

Andererseits führt die vermehrte Bildung von Keto-säuren bei Urämie zur Erhöhung organischer Säuren in der extrazellulären Flüssigkeit und zur Verstärkung der metabolischen Azidose, wenn nicht gleichzeitig der oxydative Abbau der Ketosäuren innerhalb des Trikarbonsäurezyklus intensiviert ist. Über die Umsatzgeschwindigkeit innerhalb des Zitronensäurezyklus in der Leber bei akuter experimenteller Urämie sind keine Untersuchungen bekannt. Wir bestimmten deshalb in der akut urämischen Rattenleber die Aktivität der Bernsteinsäuredehydrase. Gleichzeitig wurde der Glukoseabbau gemessen.

Methode. Der Einfluss der urämischen Stoffwechselstörung auf den oxydativen Abbau von Bernsteinsäure durch Lebergewebe wurde mit zwei Methoden untersucht: Bernsteinsäure-Oxydation durch Lebergewebe akut urämischer Ratten a) und durch Lebergewebe gesunder Ratten nach 20stündiger Einwirkung von urämischem Rattenserum b).

a) Bestimmung des Sauerstoffverbrauchs durch Leberhomogenat nach Bernsteinsäurezusatz bei 10 normalen und 10 urämischen Ratten (48 h nach Nephrektomie; Harnstoff im Serum durchschnittlich 360 mg%). 24stündige Nahrungskarenz vor Dekapitation der Tiere. Die Leber wurde sofort in eisgekühlte 0,9% NaCl-Lösung gebracht und anschliessend mit dem Apparat von POTTER und ELVEHJEM³ homogenisiert (1 g Leber-Feuchtgewicht ad 8 ml Totalvolumen mit 0,25 M Glukoselösung). Bestimmung des Sauerstoffverbrauchs im Warburg-Apparat: Totalvolumen 2,5 ml (Homogenat 0,5 ml, Sörensenpuffer 0,33 M pH 7,35 1,5 ml, Adenylsäure 0,87% 0,1 ml, KCl 4,66% 0,1 ml, MgSO₄ · 7 H₂O 4,13% 0,1 ml, Bernsteinsäure 2,95% 0,1 ml, KOH 6 n 0,1 ml im Gefässzentrum). Milieu: Luft. Dauer 1 h bei 37°C. Berechnung: mm³ O₂/g Trockengewicht/h. Sauerstoffverbrauch der Normalratten = 1.

b) Homogenisierung der Leber von 10 normalen Ratten wie bei a). 0,3 ml Serum akut urämischer Ratten wurden 0,5 ml Homogenat zugesetzt (Kontrollen mit Normalserum). 20 h bei 0°C. Bestimmung des Sauerstoffverbrauchs wie bei a); anstatt 1,5 ml Sörensenpuffer 1,2 ml Sörensenpuffer + 0,3 ml Serum. Sauerstoffverbrauch der Kontrollen = 1.

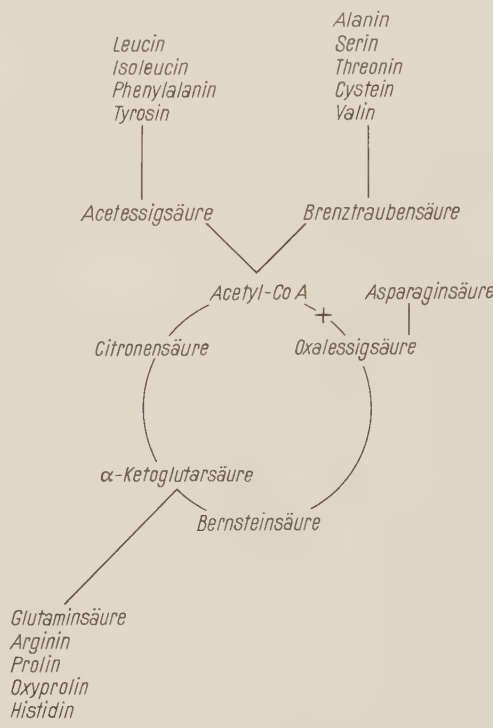
Der Glukose-Abbau durch Leberhomogenat wurde mit der Sauerstoffaufnahme gemessen. Methode wie bei a); anstatt 0,1 ml Bernsteinsäure gleiche Menge Sörensenpuffer. Werte der Normalratten = 1.

Resultate (Tab.): Signifikante Steigerung der Bernsteinsäure-Oxydation in Lebern akut urämischer Ratten und in Normalrattenlebern nach 20stündiger Einwirkung von urämischem Rattenserum.

Durchschnittliche Abnahme des Glukose-Abbaus in der akut urämischen Rattenleber; signifikante Reduktion der Glukose-Oxydation in Lebern normaler Ratten nach 20stündiger Einwirkung von urämischem Rattenserum.

Diskussion. Durch Desaminierung und Transaminierung von Aminosäuren entstehen Ketosäuren, die via Zitronensäure-Zyklus zu Kohlensäure und Wasser abgebaut werden (Abb.). In einer früheren Arbeit wurde gezeigt, dass Desaminierung und Transaminierung von DL-Alanin durch Lebergewebe akut urämischer Ratten verglichen mit Kontrollen gesteigert sind². Kontrolluntersuchungen

ergaben, dass sowohl L- als auch D-Alanin durch urämische Lebern stärker desaminiert werden. In der Leber akut urämischer Ratten ist demnach die Wirkung von L- und D-Aminosäureoxydasen im Vergleich zu Normaltieren erhöht. Beide Fermente sind stereochemisch spezifisch. Dagegen sind für die meisten L- oder D-Aminosäuren keine spezifischen Oxydasen bekannt. Die D-Aminosäureoxydase greift ausser Glykokoll, Lysin, Serin und Glutaminsäure alle D-Aminosäuren an⁴. Nach GREEN *et al.*⁵ werden folgende L-Aminosäuren durch die L-Aminosäureoxydase desaminiert: Leucin, Methionin, Norleucin, Norvalin, Phenylalanin, Tryptophan, Isoleucin, Tyrosin, Cystin, Valin, Histidin und Alanin.



Verbindungen zwischen Aminosäurenabbau und Zitronensäurezyklus nach einem Schema von BESSEY⁶

Steigerung der oxydativen Desaminierung von Aminosäuren führt zu Vermehrung der entsprechenden Keto-säuren, die im urämischen Organismus retiniert werden. Dadurch wird die metabolische Azidose (Kussmaul'sche Atmung) verstärkt; ferner vermutet man ursächliche Beziehungen zwischen Vermehrung organischer Säuren im Serum und anderen Urämiesymptomen.

Unsere Untersuchungen ergaben Steigerung der Bernsteinsäure-Oxydation durch Lebergewebe akut urämischer Ratten. Möglicherweise werden im aktivierten Zitronensäurezyklus Ketosäuren, die infolge vermehrter Aminosäurendesaminierung und renaler Retention erhöht sind, vermehrt zu Kohlensäure und Wasser oxydiert. Dadurch werden toxische Wirkungen der Ketosäuren vermindert. Die gebildeten Abbauprodukte belasten die Nierenfunktionen nicht, da sie pulmonal ausgeschieden werden.

Ob bei chronischer experimenteller Urämie dieselben Stoffwechselveränderungen auftreten, ist nicht bekannt.

⁴ K. LANG, *Der intermediäre Stoffwechsel* (Springer, Berlin-Göttingen-Heidelberg 1952).
⁵ D. E. GREEN, V. NOCITO und S. RATNER, *J. biol. Chem.* **148**, 461 (1943).
⁶ O. A. BESSEY, *J. Amer. med. Ass.* **164**, 1224 (1957).

³ V. R. POTTER und A. ELVEHJEM, *J. biol. Chem.* **114**, 195 (1936).

Bernsteinsäureoxydation und Glukoseabbau in Lebern normaler und akut urämischer Ratten und in Normalrattenlebern nach 20stündiger Einwirkung von Normal- oder Urämieserum.
Werte der Kontrollen = 1

Methode	Untersuchungs-material	Resultate	Signifikanz
Bernsteinsäureoxydation	Leber: Normalratten	1,0	$p < 0,01$
	akut urämische Ratten	1,14	
	Normalrattenleber nach 20stündiger Einwirkung von Normalserum	1,0	$p < 0,001$
	Urämieserum	1,12	
Glukoseoxydation	Leber: Normalratten	1,0	$p > 0,3$
	akut urämische Ratten	0,93	
	Normalrattenleber nach 20stündiger Einwirkung von: Normalserum	1,0	$p < 0,001$
	Urämieserum	0,84	

Vermutlich werden Leberfunktionen durch toxische biogene Amine, Xanthoproteinsubstanzen, Azidose, Elektrolyt- und Wasserhaushalt-Störungen, Anämie und Vitaminmangel bei chronisch-urämischen Zuständen geschädigt. Zum Beispiel könnte die Bildung biogener Amine zunehmen, da Aminosäuren bei abnehmendem Sauerstoffgehalt der Gewebe vermehrt dekarboxyliert werden.

Der Glukose-Abbau war in der Leber akut urämischer Ratten nicht signifikant, in Lebergewebe, das 20 h mit Urämieserum in Kontakt war, signifikant vermindert. Die Aktivierung des Zitronensäurezyklus kann deshalb nur auf Steigerung von Fettsäuren- und/oder Aminosäureabbau zurückgeführt werden.

H. THÖLEN

Medizinische Universitätsklinik Basel, 22. Juli 1959.

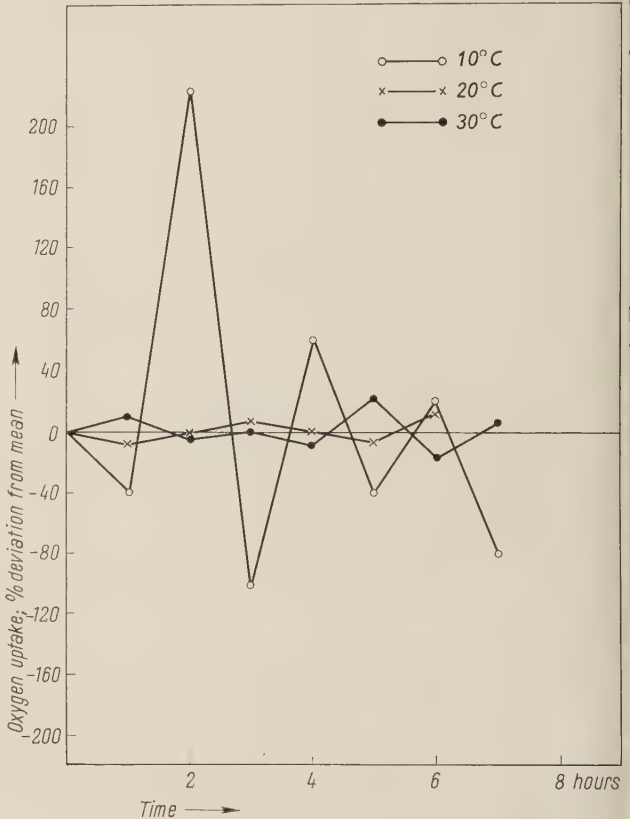
Summary

The oxydative desamination of amino acids in the liver of acute uremic rats is increased. Simultaneously the citric acid cycle in the liver of these rats is stimulated. It is suggested that the ketone-acids formed by the increased amino acid degradation are removed by the activated citric acid cycle.

Oscillatory Respiration
in *Balanus amphitrite* Darwin

Attention has been drawn by GRAINGER¹ to the fact that a sudden rise or fall in temperature, following upon maintenance under constant conditions, leads to an over-

shoot in the oxygen consumption of a number of crustaceans. This overshoot takes the form of a damped oscillation leading eventually to a steady value which is typical of the new temperature. He detected no such oscillations in carbon dioxide output, and the respiratory quotient must, therefore, fluctuate with oxygen consumption. He points out that the phenomenon of overshoot may be explained if the organism is considered as a steady state system and if, on transference to the new conditions, there is a temporary accumulation or deficiency of metabolic substances before the appropriate steady state is established. Oscillatory metabolic changes are also known to occur in the general adaptation response that follows shock and these lead either to a state of exhaustion (persistent shock) or to a state of resistance (counter shock).



Oxygen uptake of *Balanus amphitrite* as percentage deviations from means.

Attention is here drawn to the oscillatory character of the oxygen consumption, following a reduction in temperature, in *Balanus amphitrite*. The animals were taken from the Salton Sea, Southern California, where there is a dense population on all submerged material. The 'Sea' although extremely saline² (33‰) is not marine and has a somewhat different ionic composition from that of sea water. The animals were maintained in Salton Sea water at the prevailing temperature of 20–25°C between collection and the experiments. The experiments, which formed part of a large series, were made on isolated animals detached from their calcareous shells by cutting through the small muscles attaching the body to the opercular valves; injury was slight and had no detectable effect on the results (see BARNES and BARNES³ for further details).

² L. H. CARPELAN, Limnol. Oceanogr. 3, 373 (1958).
³ H. BARNES and M. BARNES, Veröff. Inst. Meeresforsch. Bremerhaven (in press).

¹ J. N. R. GRAINGER, Nature 178, 930 (1956).

Oxygen uptake was determined by the standard Warburg technique the animals being maintained in Salton Sea water with no addition of substrate: 10 mg/l chloromycetin was added to inhibit bacterial action. Readings were taken at hourly intervals.

Typical results are shown in the Figure. Since the mean oxygen uptake varies with temperature, the percentage deviations from the mean values over the whole period are given. The animals on which the results are quoted were large and of similar size; oxygen uptake is weight dependent, but no difference in relative behaviour was found with the different size groups. It is evident that while there is some variation in the oxygen uptake from hour to hour at 20° and 30°C, the changes are small compared with the hourly fluctuations when the animals are held at 10°C; these take the form of a damped oscillation. A large number of species of barnacles have been investigated under a wide variety of experimental conditions and marked oscillations of this type and magnitude have only been found in this species. (It must be pointed out that small oscillations particularly if they were of a short period would require a different technical approach.)

In intertidal species which are subjected to rapid changes of temperature in their natural habitat, the metabolic processes might be expected to be so coupled as to reduce the oscillatory behaviour, whether it is overshoot or response to shock. It is, however, not so evident why other sub-littoral species which live in a comparatively constant environment should not show a similar behaviour when subjected to equally large temperature changes. It is perhaps, therefore, of significance that *B. amphitrite* was the only warm water and tropical sub-littoral species investigated. The fact that northern forms when subjected to a rise in temperature do not behave in an oscillatory manner comparable to that of this southern form when subjected to similar falls in temperature suggests that the coupling of the metabolic processes may be different in the two types of species; further work on this line would perhaps help to elucidate some of the factors concerned in determining their respective distribution.

H. BARNES and MARGARET BARNES

The Marine Station, Millport (Scotland), April 20, 1959.

Résumé

Chez *Balanus amphitrite*, espèce des eaux chaudes, l'utilisation de l'oxygène par des individus isolés (extraits de leur coquille), soumis à une température peu élevée est de forme oscillatoire, contrairement à ce que l'on observe chez des espèces des eaux froides. Il se peut que cette différence caractérise de manière générale deux types d'espèces.

Action of Insulin *in vitro* on the Glucose Uptake of the Spinal Cord of the Rat*

It is not yet clear whether insulin has any effect on the metabolism of glucose through the nervous system or not¹. Recently RAFAELSEN² published his results, accord-

Table I

Action of Insulin on the Glucose Uptake and Oxygen Consumption of the Spinal Cord. Krebs Buffer. Not anaesthetised

	Mean	<i>t</i>	<i>P</i>	No. experiments
Glucose uptake mg × 100 mg of tissue				
Insulin 10 ⁻¹ . .	0.236	0.000	> 0.9	8
Control	0.236			8
Oxygen consumption microlitres × 100 mg of tissue × h				
Insulin 10 ⁻¹ . .	13.4	0.209	0.8 < <i>P</i> < 0.9	8
Control	13.5			8

ing to which the spinal cord of the rat appears to be sensitive to this hormone. Since most authors³, as also we ourselves⁴, were unable to find any effect of insulin on the nervous system, we thought it interesting to repeat RAFAELSEN's test in order to try to confirm his results.

Methods. We utilised rats with a body weight of 100 to 160 g, fasted for 24 h. The extraction of the spinal cord was made following exactly the same method as RAFAELSEN², after a previous anesthetic by inhalation of a mixture of 50% CO₂/O₂. In some other groups of experiments, the rats were decapitated in order to elucidate the effect, if any, of the anesthetic.

The medullary portion was divided into two parts weighting 100–120 mg, their weight being determined through the weighting differences of the incubation flasks. These pieces were directly incubated in 2 cm³ of the Gey and Gey (RAFAELSEN) or Krebs medium according to the cases. For this purpose, Warburg glasses with a total volume of 15 cm³ were employed and the incubation (Warburg S. L.) was made at a temperature of 37.5°C for 120 min, at 80–90 oscillations/min and 4 cm amplitude. The flasks were aerated with a mixture of 95% oxygen and 5% carbon dioxide, for 1 min. Glucose was determined by the glucose-oxidase method, with a maximum development of colour. The insulin employed was Lilly⁵, free from glucagon, at a concentration of 10⁻¹ I. U. per cm³.

The glucose uptake was calculated from the decrease of glucose level in the medium during the incubation and expressed as: mg glucose per 100 mg of tissue (spinal cord) per 60 min. The determination of the consumption of oxygen is expressed in microlitres consumed per 100 mg of tissue per 60 min. Student's 't' test was used for statistical analysis of the data.

Results. Table I contains the results obtained in tests made with rats decapitated utilising the Krebs buffer. There are no differences in the glucose consumption between the series treated with insulin and the control animals. There are also no differences in oxygen consumption. There is no sensitiveness to insulin.

Tests reproduced in Table II were made with rats decapitated utilising the same buffer as RAFAELSEN (Gey and Gey). The difference is not significant, either in the consumption of glucose or in that of oxygen. There is no sensitiveness to insulin.

* Preliminary communication.
¹ H. E. HIMWICH, *Brain Metabolism and Cerebral Disorders* (The Williams & Wilkins, 1951). – D. RICHTER, *Metabolism of the Nervous System* (Pergamon Press, 1957). – H. McILWAIN, *Biochemistry and the Central Nervous System* (J. & A. Churchill, 1955). – A. E. RENOLD, J. ASHMORE, and A. B. HASTINGS, *Vitamins and Hormones* 14, 170 (1956).
² O. J. RAFAELSEN, *Lancet* 1958, 941.

³ C. P. PARK, L. H. JOHNSON, J. H. WRIGHT, and H. BATSEL, *Amer. J. Phys.* 191, 13 (1957).
⁴ D. MARTIN-HERNANDEZ, R. R-CANDELA, and J. L. R-CANDELA, *Rev. iber. Endocrin.* 5, 39 (1958).
⁵ We are indebted to Dr. W. R. KIRTLEY for the generous supply of this hormone.

Table II

Action of Insulin on the Glucose Uptake and Oxygen Consumption of the Spinal Cord. Buffer Gey and Gey. Not anesthetised

	Mean	σ	t	P	No. experiments
Glucose uptake mg $\times$ 100 mg of tissue					
Insulin 10^{-1}	0.328	0.027	1.867	$0.05 < P < 0.1$	14
Control . .	0.304	0.033			14
Oxygen consumption: microlitres $\times$ 100 mg of tissue $\times$ h					
Insulin 10^{-1}	12.3	1.06	1.1211	$0.2 < P < 0.3$	14
Control . .	12.7	0.65			14

In Table III the results are obtained by exactly following RAFAELSEN's technique: that is to say, after previously anesthetising the rat with a mixture of 50% CO_2/O_2 . In this kind of test, the spinal cord appears sensitive to the action of insulin because the difference in glucose consumption of the tissue with and without insulin is significant. There is, however, no significant difference in the oxygen consumption.

Table III

Action of Insulin on the Glucose Uptake and oxygen Consumption of the spinal Cord. Buffer Gey and Gey. Anesthetised with a CO_2/O_2 mixture at 50%

	Mean	σ	t	P	No. experiments
Glucose uptake: mg $\times$ 100 mg of tissue					
Insulin 10^{-1}	0.485	0.021	3.8693	< 0.01	9
Control . .	0.290	0.029			9
Oxygen consumption: microlitres $\times$ 100 mg of tissue $\times$ h					
Insulin 10^{-1}	17.1	2.07	0.5263	$0.6 < P < 0.7$	8
Control . .	16.6	1.38			8

Discussion. We were able to confirm RAFAELSEN's results, although our own data give them a different interpretation, because we observed that the spinal cord is *only sensitive* to insulin when the rat has been previously anesthetised with CO_2 . The basal glucose-uptake is the same in the experiments but in the test made without anesthesia, no effect is produced when adding this hormone. Similar results have obtained URELES and MURRAY⁶, because they have found that r. c. b. uptake of I^{131} triiodothyronine increases either with marked CO_2 retention (patients) or when the blood has been bubbled with CO_2 into the flask *in vitro*.

J. L. R-CANDELA and
D. MARTIN-HERNANDEZ

Instituto de Metabolismo y Nutrición, Madrid (Spain),
June 9, 1959.

Résumé

La moelle épinière ne paraît pas sensible à l'insuline *in vitro* si elle provient d'un animal décapité alors qu'elle l'est de façon significative ($P < 0,01$) si l'animal était anesthésié avec un mélange de CO_2/O_2 à 50%.

⁶ A. URELES and M. MURRAY, cited by M. W. HAMOLSKY *et al.*, J. clin. Endocrin. Met. 19, 103 (1959).

Ion Adsorption and Excitation II

It has been shown in a previous communication¹ that the internal and external ion concentrations of nerve can be related to the net quantity of ions fluxing during the passage of a single impulse by the expression $f = A \nu (C_i - C_o)$. It was also deduced that the ions involved in these fluxes form a monolayer at the nerve surface and that they are not hydrated. This was taken as an indication that excitation may be accompanied by an adsorption-desorption process.

The quantity $A \nu$ has been defined as that volume of the nerve substance in which the ionic concentration corresponds to that of the nerve interior or to that of the bathing solution depending on the presence or absence of the flux quantity.

The advantage of this formulation is that by summing the flux values over all such unit volumes available, the gross ion concentration difference between the axoplasm and the bathing fluid can be expressed in terms of the flux quantity characteristic of a single discharge.

Since $A \nu$ can also be regarded as a sort of 'extended surface', being in fact only one half of an ion thick and situated presumably at the functional surface of the nerve, this surface may be considered as having the same ionic composition as the nerve interior. This suggests that the forces which operate in maintaining the ionic composition of the nerve interior are identical with those which maintain the ionic composition of the nerve surface. Conveniently these forces may be characterized as adsorption forces, opposite and equal in magnitude to the potentials as given for each ionic species by the relation $E = kT/e \ln (C_i/C_o)$.

It follows from such a view that since the surface monolayers of the major monovalent ions can be regarded as spatially superimposed, the resting potential of the cell may be assumed to correspond to the algebraic sum of the potentials due to each of the major ions represented at the surface. This leads to the expression

$$R.P. = kT/e \ln (K_i/K_o \times Na_i/Na_o \times Cl_o/Cl_i).$$

Taking the external concentrations of K and Na ions as $K_o = 22 \text{ mM/kg}$ and $Na_o = 440 \text{ mM/kg}^2$ and the internal ones as $K_i = 345.3 \text{ mM/kg}$ and $Na_i = 62.5 \text{ mM/kg}^1$, and using the value of 59 mV for the corrected resting potential of the squid giant axon³, one obtains from the above formula the ratio $Cl_o/Cl_i = 4.5$.

According to the data of BEAR and SCHMITT⁴ this ratio is about 4, the agreement between this value and the one calculated above suggesting that the assumption involved in the formulation is essentially correct.

I wish to thank Dr. B. W. ZWEIFACH for his kind advice and interest.

E. ASCHHEIM

Department of Pathology, New York University, Bellevue Medical Center, New York, May 25, 1959.

Résumé

Une façon simple de formuler le potentiel de repos de l'axone géant du Calmar est présentée. L'équation est

¹ E. ASCHHEIM, Science 129, 779 (1959).
² A. L. HODGKIN, Biol. Rev. Cambridge Phil. Soc. 26, 339 (1951).
³ A. L. HODGKIN and B. KATZ, J. Physiol. 108, 37 (1949).
⁴ R. S. BEAR and F. O. SCHMITT, J. cell. comp. Physiol. 14, 205 (1939).

fondée sur une démonstration antérieure prouvant que pendant l'excitation les cations importants forment une seule couche à la surface fonctionnelle du nerf. La formule est employée pour calculer la distribution du chlorure. Le résultat est en accord avec les valeurs expérimentales publiées.

Effects of Chronic Lesions of the Peduncles on Cerebellum Cholinesterase Activity, in the Albino Rat

In a previous paper¹, the high true cholinesterase activity of cerebellum – predominantly localized in cerebellar cortex – has been related to cerebello-petal tracts, with the tentative purpose of applying available information on the ACh system in cerebellum to a cholinergic mechanism of afferent fibres. The hypothesis was supported by: (a) the differential distribution of true cholinesterase over the structurally uniform cerebellar cortex; (b) the sensitivity of some intracortical neurons to acetylcholine; (c) the presence of cholineacetylase in some cerebellar afferent systems.

The present paper concerns the fate of cerebellum cholinesterase after unilateral lesions involving, more or less extensively, cerebellar peduncles.

Adult albino rats of the Wistar strain were used throughout. Surgical procedure was performed aseptically, under nembutal anesthesia. Peduncles were approached from the IV ventricle, the extent and the location of the lesion being reconstructed on necroscopic examination. In a few animals only the opening of the IV ventricle was performed (sham operation).

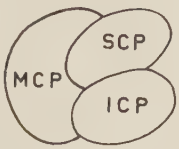
Estimations of enzyme activity were carried out on the 10th day following the operation, by the Warburg manometric method, as previously described¹. The two halves of the cerebellum of operated animals were tested separately, while cerebella of controls were tested *in toto*, having previously stated the same level of activity in the two halves. All estimations were carried out in duplicate, using acetyl- β -methylcholine and butyrylcholine as 'specific' substrates for true (ChEI) and pseudo-(ChEII) cholinesterase, respectively. Fresh solutions of substrates were used in each experiment, the final concentrations being acetyl- β -methylcholine 0.03 M and butyrylcholine 0.01 M. Results are expressed as μ l of CO₂ evolved/h/g of wet weight of tissue.

The Table gives the results – both as absolute values and as per cent fall in activity – of seven animals out of a total of twenty operated. Results were consistent for similar types of lesions, and only the typical examples have been reported so as to summarize the course of events during progressive change of location and extent of peduncular lesion. Values refer to ChEI, no significant variation having ever been found in ChEII activity.

Unilateral lesions of cerebellar peduncles are followed by a fall in cerebellum ChEI content, the value depending on the extent and location of the lesion and reaching its maximum when all three peduncles are severed. Maximal drops in activity range around 60% in the ipsilateral side and 40% in the controlateral one, each peduncle contributing to a certain extent, as roughly indicated by results of partial lesions.

¹ L. SPERTI, S. SPERTI, and P. ZATTI, Arch. ital. Biol. sper. in press.

Table
True cholinesterase activity of rat cerebellum after unilateral lesions involving cerebellar peduncles

	Homolateral half		Controlateral half		Control (<i>in toto</i>)
	Q MeCh	%	Q MeCh	%	Q MeCh
Sham operation	1014		1008		994
A 	869	– 13	988	– 1	995
B 	785	– 24	1044	0	1030
C 	874	– 22	1024	– 8	1119
D 	468	– 52	860	– 12	977
E 	598	– 42	880	– 15	1030
F 	487	– 50	817	– 16	976
G 	388	– 63	635	– 39	1042

ICP = inferior cerebellar peduncle; MCP = middle cerebellar peduncle; SCP = superior cerebellar peduncle

Q MeCh = μ l CO₂/g wet weight of tissue/h with acetyl- β -methylcholine as substrate; % = fall in activity as per cent of the control tested in the same experiment, which was of the same litter of the operated animal

Figures for sham operation are the mean of three experiments. Drawings roughly indicate location and extent of lesions

The disappearance of ChEI – in cerebellum predominantly localized in the cortex¹⁻⁴ – is clearly related to the degeneration of afferent fibres (partially crossed), the efferent ones – almost entirely nuclear in origin – being out of question.

As to the behaviour of ChEI in Wallerian degeneration, afferent tracts to cerebellum seem then to resemble pre-

² A. S. V. BURGEN and L. M. CHIPMAN, J. Physiol. 114, 296 (1951).

³ S. C. SHEN, P. GREENFIELD, and E. J. BOELL, J. comp. Neurol. 102, 717 (1955).

⁴ G. B. KOELLE, J. comp. Neurol. 100, 211 (1954).

gangliar sympathetic⁵ rather than peripheral motor fibres⁶, the ChEI concentrated at the synaptic level being extensively involved as indicated by the high drops in activity.

The dependence of cortico-cerebellar ChEI activity from the integrity of afferent fibres closely agrees with histochemical data of a selective distribution of the enzyme in the cortico-cerebellar layers containing afferent terminals³⁻⁴. Both facts fit in with the above-mentioned information to support a possible cholinergic mechanism of some cerebellar afferent systems.

L. SPERTI and S. SPERTI

Istituto di Fisiologia e Istituto di Chimica Biologica dell'Università di Padova, June 18, 1959.

Riassunto

Lesioni unilaterali nell'ambito dei peduncoli cerebellari sono seguite, nel ratto, da una caduta dell'attività colinesterasica vera del cervelletto, che raggiunge i valori massimi del 60% nella metà ipsilaterale e del 40% in quella controlaterale nel caso di sezione totale dei tre peduncoli. In nessun caso l'attività della pseudo-colinesterasi è risultata significativamente modificata.

⁵ C. H. SAWYER and W. H. HOLLINSHEAD, *J. Neurophysiol.* 8, 137 (1945).
⁶ R. COUTEAUX, *Int. Rev. Cytol.* 4, 335 (1955).

Effects of Midline Cerebellar Splitting and of Lesions in Cerebral Cortex on Cerebellum Cholinesterase Activity, in the Albino Rat

Chronic unilateral section of rat cerebellar peduncles has been shown¹ to reduce true cholinesterase activity (ChEI) in both homolateral and controlateral halves of cerebellum, drop values ranging around 60% and 40% respectively. Results have been referred to the degeneration of afferent fibres ending in cerebellar cortex – where ChEI is highly concentrated²⁻⁵ –, a partial intracerebellar crossing⁶ possibly accounting for the controlateral effect.

Partial or total mid-sagittal division of cerebellum – involving only the fibres crossing the midline – has now proved to give, in the two halves of cerebellum, balanced drops of ChEI activity, approaching, when division is complete, the fall value previously obtained in the controlateral side of cerebellum. As in the case of lesions of cerebellar peduncles, no significant variation was ever found in pseudocholinesterase (ChEII) activity. Results, schematically represented in Table I, refer then only to ChEI, and are in each case the mean of the values obtained on the two halves of cerebellum, which never differed more than 3%.

¹ L. SPERTI and S. SPERTI, *Exper.* 15, 441 (1959).
² A. S. V. BURGEN and L. M. CHIPMAN, *J. Physiol.* 114, 296 (1951).
³ S. C. SHEN, P. GREENFIELD, and E. J. BOELL, *J. comp. Neurol.* 102, 717 (1955).
⁴ L. SPERTI, S. SPERTI, and P. ZATTI, *Arch. ital. Biol. sper.*, in press.
⁵ G. B. KOELLE, *J. comp. Neurol.* 100, 211 (1954).
⁶ J. JANSEN and A. BRODAL, *Aspect of Cerebellar Anatomy* (J. Grundt Tanum Forlag, Oslo 1954), p. 83.

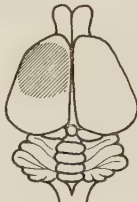
Table I
ChEI activity of rat cerebellum following mid-sagittal division

		
QEX 879 QCO 985	-11% QEX 918 QCO 1080	-33% QEX 653 QCO 976

Drawings outline the extension of the lesion

QEX, Qco = ChEI activity of the operated animals and of the controls, expressed as $\mu\text{l CO}_2/\text{g wet weight of tissue/h}$, with acetyl- β -methylcholine as substrate. Per cent falls in activity are also reported. In each experiment, the control was of the same litter as the operated animal

Table II
ChEI activity of rat cerebellum following more or less extensive ablations of cerebral cortex

		
QEX 1120(R) 0% 1145(L) +2%	QEX 1010(R) -1% 979(L) -4%	QEX 1150(R) +1% 1195(L) +5%
QCO 1125	QCO 1018	QCO 1135

Drawings outline the cortical lesion

QEX, Qco = as defined in Table I. R and L between brackets indicate the right and left half of cerebellum

The drop in ChEI activity is only the consequence of the degeneration of afferent terminals in cerebellar cortex. Deafferentation of pontine neurons, relaying impulses from cerebral cortex, no longer reproduces the effects of section of middle cerebellar peduncle, containing ponto-cerebellar axons. Table II schematically represents the results of more or less extensive ablations of cerebral cortex: no variation in either ChEII or ChEI activity has ever been found in the two halves of cerebellum.

L. SPERTI and S. SPERTI

Istituto di Fisiologia e Istituto di Chimica Biologica dell'Università di Padova, June 18, 1959.

Riassunto

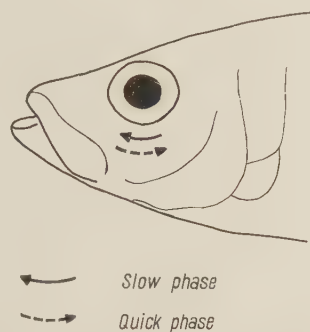
La divisione sagittale mediana del cervelletto è seguita, nel ratto, da una eguale diminuzione della attività colinesterasica vera nelle due metà del cervelletto, che raggiunge, nei casi di divisione completa, valori che si avvicinano a quelli osservati nella metà controlaterale per sezione totale, unilaterale, dei peduncoli.

Lesioni della corteccia cerebrale, anche se estese, non modificano l'attività colinesterasica vera del cervelletto.

In nessun caso vennero osservate modificazioni dell'attività pseudocolinesterasica.

A Peculiar Nystagmus and a Corresponding Foveal Structure in the Eye of the Herring (*Clupea harengus* L.)

In herring swimming in aquaria (the techniques developed for the capture, transport, and maintenance of these particularly delicate fishes were described in previous papers¹) we observed a peculiar type of eye movement. While a fish is swimming forward, the eyeball rotates around an axis through the pupil and about at right angles to the plane of the pupil. When a fish passes the observer from the right to the left, the left eye shows alternately a slow clockwise rotation and a rapid jerk-like anti-clockwise rotation (Figure).



A peculiar nystagmus in the eye of the herring (*Clupea harengus* L.).

These movements are highly reminiscent of nystagmic movements. Sometimes the slow phase of an eye nystagmus is a compensatory or adjusting movement by which the image of an object is kept on an area of the photosensitive surface with a highly developed resolving power.

The nystagmic movements described above are thus suggestive of such an area at the ventral side of the retina of the herring's eye. This would be in accordance with the feeding behaviour of this fish. The prey is usually captured with a visually steered switching movement from below². Moreover, the position of the lens in the eye of the herring seems to be indicative of a wide field of vision above the head of the fish³.

Histological study of the herring's eye revealed a foveal structure at the ventral side, situated immediately below the spot where the optic nerve penetrates the retina. Here the cones have a smaller cross-sectional size and they are about ten times more densely packed as compared with the rest of the retina. Rods are almost absent in this foveal region, whereas in the other parts of the retina there are some hundreds of rods to every cone. Moreover, some layers of nerve cells – especially the bipolar cells – are more highly developed in the foveal region. Similar retinal structures have been described in the eye of the sardine (*Clupea pilchardus* W.)⁴.

The origin and the function of 'optokinetic' movements have been elaborately discussed⁵. The question at issue seems to be whether the nystagmic movements observed in an actively moving animal are optokinetically released by the movements of images along the photosensitive surface of the eye, or whether they are of central origin. Since nystagmic movements are found in lower animals like arthropods as well as in vertebrates, generalizations are difficult and dangerous. Keeping this in mind it still seems useful to realize in which ways nystagmic movements can be evoked.

VON BUDDENBROCK⁶ restricted the concept of 'optomotor reactions' to responses to retinal movements of the complete image pattern of the entire visual field. He discriminated 'optomotor reactions' from the visual perception of some moving object which may release following movements of the eyes or of the whole animal. The first example of 'optomotor reactions' he gives, the human 'railway-nystagmus', reveals however the weakness of his concept, for the slow phase of the railway-nystagmus is a following movement by which the image of a definite object is kept on the fovea for some time. The movements of the peripheral retinal images of all other objects in the visual field – each image moving with its own speed in accordance with the distance between the eye and the relative object – are without effect.

The twofold character of the optokinetic nystagmus was recognized by TER BRAAK⁷, who described a 'look nystagmus' and a 'stare nystagmus'. The 'look nystagmus' was elicited by a moving object across a stationary background, while the 'stare nystagmus' was observed when the entire visual field rotated around the experimental animal. Obviously the 'stare nystagmus' is related to extra-foveal photic orientation while the 'look nystagmus' is related to detailed foveal vision⁸. In animals with some type of foveal vision, we must always reckon with the possibility that the eyes are coupled to some object in the visual field by retinal feedback, whether the movements of the retinal image of the object are the result of active movements of the animal or not. This coupling serving the visual function does not necessarily interfere with the animal's capacity to discriminate via photic information between these two types of movement. For non-foveal vision this exact coupling is not necessary because the movement of an image across a non-foveal retinal region does not impair the perception of the object so long as the speed of this movement is not too high.

It is conceivable that in organisms with poorly developed foveal structures the eye movements as a result of a kinetic component of central origin need almost no correction via retinal feedback. As far as observation and registration permits to decide, the eye nystagmus in the

⁵ J. W. G. ter BRAAK, Arch. néerl. Physiol. 21, 309 (1936). – H. MITTELSTAEDT, Naturwiss. 36, 90 (1949). – E. v. HOLST and H. MITTELSTAEDT, Naturwiss. 37, 464 (1950). – R. W. SPERRY, J. comp. Physiol. Psychol. 43, 482 (1950). – W. v. BUDDENBROCK and I. MOLLER-RACKE, Exper. 8, 392 (1952); Exper. 9, 191 (1953). – S. DIJKGRAAF, Exper. 9, 112, 387 (1953); Exper. 11, 329 (1955); Pubbl. Staz. zool. Napoli 28, 341 (1956); Z. vgl. Physiol. 38, 491 (1956). – G. THINES, Rev. Quest. scient. 1957, 430.

⁶ W. v. BUDDENBROCK, Vergleichende Physiologie, Bd. I, Sinnesphysiologie (Birkhäuser Verlag, Basel 1952), p. 87.

⁷ J. W. G. ter BRAAK, Arch. néerl. Physiol. 21, 309 (1936).

⁸ For recent dates about the fixation movements of the human eye see: R. W. DITCHBURN and B. L. GINSBURG, J. Physiol. 119, 1 (1953). – H. DRISCHER and C. LANGE, Pflügers Arch. 262, 307 (1956). – T. N. CORNSWEET, J. opt. Soc. Amer. 46, 987 (1956). – For fixation movements in insects see: H. MITTELSTAEDT, Regelungstechnik 2, 226 (1954); Rec. Adv. in Invert. Physiol., Univ. Oregon Publ. (1957), 51.

¹ F. J. VERHEIJEN, Exper. 9, 193 (1953); Pubbl. Staz. zool. Napoli 28, 225 (1956).

² F. J. VERHEIJEN, Exper. 9, 193 (1953); Pubbl. Staz. zool. Napoli 31, 146 (1959). – J. H. S. BLAXTER and F. G. T. HOLLIDAY, Mar. Res. Scott. Home Dep., 1958, No. 6.

³ J. R. BRETT in: M. E. BROWN, The Physiology of Fishes, vol. 2 (Academic Press Inc., New York 1957), p. 131.

⁴ V. VILTER, C. R. Soc. Biol. 144, 200 (1950).

actively turning lobster *Palinurus vulgaris* and in the crab *Carcinus maenas* is identical, whether the animal is blinded or not. It has been concluded that this nystagmus is not a photokinetic response but a spontaneous action of central origin with possibly a steering component originating from the perception of movements of the limbs relative to the body⁹.

It is hoped that further studies will reveal whether the function of the slow phase of the eye nystagmus described in the herring is restricted to the keeping of images on the ventral foveal region or whether it has an additional and more general function in photo orientation, and whether it is released exclusively by image movements or whether it has an active central component. In this way some contribution might be made towards a further understanding of the various aspects of 'optokinetic' movements in general. The described eye nystagmus of the herring has the advantage that the labyrinth and other receptors which might registrate rotations are precluded as sources of extra-retinal information since rotations are not involved in the genesis of this nystagmus. The delicacy of the animals, however, makes surgical interference rather precarious.

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Zusammenfassung

An Heringen, die in Aquarien schwimmen, kann ein Nystagmus beobachtet werden. Hierbei rotiert das Auge um eine Achse, die ungefähr senkrecht zur Pupillenebene steht. Der Nystagmus steht wahrscheinlich in Beziehung zu einer fovealen Struktur auf der ventralen Hälfte der Retina. Die Auslösung und Bedeutung «optokinetischer» oder «optomotorischer» Reaktionen wird diskutiert.

⁹ S. DIJKGRAAF, Pubbl. Staz. zool. Napoli 28, 341 (1956); Z. vgl. Physiol. 38, 491 (1956).

High-fat Diet and the Development of Obesity in Albino Rats

The relationship between the ratio of the main nutrients in the diet and the development of obesity in man is still the subject of extensive research¹. Among experimental

¹ J. MAŠEK, L. KŘÍKAVA, and K. OŠANCOVÁ, Int. J. prophyl. Med. 2, 132 (1958); Second Intern. Congress of Dietetics, Rome 1956, Coll. Communications, p. 271 (1958); Čs. gastroenterol. a výž. 13, 246 (1959). – K. OŠANCOVÁ, Čs. hygien.a 3, 131 (1958).

work concerned with this problem, the findings of MICKELSEN *et al.*² and of BARBORIAK *et al.*³ who described the development of obesity in rats fed for a prolonged time on a high-fat diet, deserve special attention. However, it must be recalled that the high-fat diet used in these authors' experiments had a higher energy value than the high-carbohydrate control diet and a lower percentage of energy derived from protein. It was therefore of interest to elucidate whether a high ratio of dietary fat *per se* leads to the development of obesity.

Young adult male rats (Wistar strain) were fed *ad libitum* for 44 weeks diets with different proportions of fat, carbohydrate, and protein. The diets contained the same ratio of a basal mixture; the nutrient administered in excess was provided in the form of an isocaloric amount of margarine, starch, or casein. The diets were supplemented by a 2.5% solution of agar to make them not only isocaloric (1 g = 2.3 cal), but also roughly isovoluminous. Except for the nutrient given in excess, the ratio of energy derived from the remaining two nutrients was practically equal (Table). These diets are a slight modification of the diets described in a previous work⁴. The rats were weighed once a week and the food intake of the different groups was measured. After 44 weeks on the experimental diets the animals were killed and the fat content of the whole eviscerated carcass was estimated by the method used by COHN *et al.*⁵.

The results of the experiment are summarized in the table. We can see that the animals fed the high-fat and high-carbohydrate diet do not differ significantly in the weight increments nor in the amount of body fat. The growth of both groups was harmonious and the animals did not develop any signs of deficiency. In agreement with data in the literature⁶, the weight increment and body fat were reduced in the group that were fed an excess of casein. The food intake per animal in all three experimental groups was practically equal. The caloric efficiency of the high-fat and the high-carbohydrate diet was thus the same while that of the high-protein diet was considerably lower. It must be mentioned that identical results

² O. MICKELSEN, S. TAKAHASHI, and C. CRAIG, J. Nutr. 57, 541 (1955).
³ J. J. BARBORIAK, W. A. KREHL, G. R. COWGILL, and A. D. WHEDON, J. Nutr. 64, 241 (1958).
⁴ R. PETRÁŠEK and P. FÁBRY, Arch. int. Physiol. Biochim. 66, 610 (1958).
⁵ C. COHN, D. JOSEPH, and E. SHRAGO, Metabolism 6, 381 (1957).
⁶ P. F. FENTON and C. J. CARR, J. Nutr. 45, 225 (1951). – P. F. FENTON and M. T. DOWLING, J. Nutr. 49, 319 (1953). – E. FALTOVÁ and O. POUPA, Čs. gastroenterol. a výž. 10, 229 (1956).
⁷ P. FÁBRY, P. HAHN, O. KOLDOVSKÝ and J. MAŠEK (in preparation).

Table
Influence of Different Diets on Weight Gains and Body-Fat of Albino Rats after 44 Weeks of the Experiment

Group	Composition of diet			No. of animals	Initial weight (g)	Final weight (g)*	Weight gain (g)*	Fat content of eviscerated carcass (%)*
	Cal. % protein	Cal. % fat	Cal. % carbo-hydrate					
High-fat	14.9	70.0	15.1	14	191 ± 6.5	447 ± 18.2	256 ± 16.0	20.45 ± 1.23
High-carbohydrate	15.2	10.5	74.3	13	189 ± 4.4	421 ± 18.3	232 ± 13.6	19.88 ± 1.58
High-protein	73.1	11.5	15.4	14	191 ± 6.9	314 ± 12.1	123 ± 10.1	10.69 ± 0.99

The values are given in the averages of the groups (weights as the nearest whole number) ± S.E.
* The values of the high-fat and high-carbohydrate group do not differ significantly. The difference between the high-protein group and the remaining two groups is statistically significant for *P* < 0.01.

were obtained in a preliminary experiment with groups of 7, 6, and 8 rats, having initial weights of ca. 90 g and fed these contrasting diets for 20 weeks.

The results of our experiments indicate clearly that the high ratio of dietary fat *per se* did not lead to the development of obesity in young adult rats. In subsequent experiments we revealed that a high-fat diet does not produce obesity in Wistar rats even when administered from the 18th or 30th day after birth, respectively⁷. In the experiments of MICKELSEN *et al.*² and BARBORIAK *et al.*³, other factors in addition to the percentage of calories provided by fat must have been at play which led to an absolute or relative hyperphagia and finally to obesity of the experimental animals. For instance, the high-fat diet used in their experiments contained more calories (per weight or volume) than the control diet and the energy value derived from protein was substantially smaller. It is also possible that the strains used by these workers (Osborne-Mendel and Sprague-Dawley resp.) respond to the high-fat diet in a different manner.

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Institute of Human Nutrition, Prague (Czechoslovakia), July 15, 1959.

Zusammenfassung

Männliche, geschlechtsreife Wistar-Ratten wurden 44 Wochen lang mit isokalorischen Diäten gefüttert, die entweder einen erhöhten Fett- oder Kohlehydrat- bzw. Eiweissanteil enthielten. Zwischen den eine fettreiche und eine kohlehydratreiche Diät erhaltenden Tieren zeigten sich keine statistisch bedeutsamen Unterschiede in bezug auf ihre Gewichtszunahme und den Gesamtanteil des Körperfettes. Diejenigen Tiere, die eine Diät mit erhöhtem Kaseingehalt erhielten, nahmen in Übereinstimmung mit den Literaturangaben weniger an Gewicht zu und lagerten auch weniger Körperfett ab. Wie aus unseren Ergebnissen hervorgeht, muss ein hoher Fettanteil in der Diät von sich aus noch nicht zur Fettsucht bei Ratten führen.

The Specific Gravity of Liver and its Relation to the Fat Content following High Fat Diets and Carbon Tetrachloride Poisoning

Fat is a normal constituent of all livers, and its concentration is increased in many diseases and metabolic

changes, e.g. in toxic hepatitis, cirrhosis, spontaneous diabetes mellitus, and acetonemia. Parasitic infections can cause an increase in the amount of fat but this may be more localised than in toxic hepatitis. During an investigation by a colleague (Dr. J. S. WILKINSON) on spontaneous diabetes in dogs, consideration was given to a routine and rapid estimation of fat in biopsy and small *post-mortem* samples. Several workers have investigated chemical methods notably BILLING, CONLON, HEIN, and SCHIFF¹ who have described a microtechnique and its application to the investigation of human liver disease. Although this method is elegant, it requires rather specialised and skilled techniques, so it was decided to investigate the possibility of establishing a relation between the specific gravity of a liver sample and its fat content. Preliminary experiments indicated that *fresh* liver samples could be titrated with benzene and chloroform^{2,3} to give fairly precise measurements of their specific gravities with little loss of lipid material into the solvents during the process. This led to the planning of two major experiments. In the first a group of thirty mice were fed *ad libitum* quantities of a butter fat diet containing 3% of a salt mixture⁴ for 4–5 days. Each day five animals were sacrificed by light etherisation and exsanguination, and the livers removed. These were weighed rapidly in air, in benzene and then titrated with chloroform. Three small portions (5–20 mg) were removed at random and also titrated. The portions of liver were combined, dried (100–110°C, 18 h) weighed, and extracted several times with a 50:50 mixture of diethyl ether: petroleum ether (B. P. 40–60°C) to determine the fat content. It was found that significant regressions could be extracted from the *probit* per cent fat per dry weight of the liver and the specific gravities determined by the three methods. Further, it was found that there was no significant difference between the three regressions (Table I). Regressions on group values gave similar results (Table II).

The gradient diffusion method for specific gravity measurement was also investigated. This gave similar results. In preparing a gradient of suitable range mixtures of bromobenzene and charcoal decolourised domestic

¹ B. H. BILLING, H. J. CONLON, D. E. HEIN, and L. SCHIFF, *J. clin. Invest.* 32, 214 (1953).
² D. G. HARVEY, *Brit. Vet. J.* 113, 52 (1957).
³ D. M. G. ARMSTRONG and A. E. HAWKINS, *Physics Biol. Med.* 2, 338 (1958).
⁴ R. B. HUBBELL, L. B. MENDEL, and A. J. WAKEMAN, *J. Nutr.* 14, 273 (1937).

Table I
Regressions (*b*) of probit per cent liver fat (dry weight) and specific gravity. Feeding experiments

Method	<i>n</i>	<i>b</i> ± S. D.	<i>P</i>	Equation
1. Weighing Air/Benzene	25	−0.0180 ± 0.0014	< 0.001	<i>y</i> = 4.76 − 0.018 <i>x</i>
2. Titration: Whole liver	25	−0.0144 ± 0.0090	< 0.001	<i>y</i> = 4.59 − 0.0144 <i>x</i>
3. Titration: Mean three pieces .	25	−0.0162 ± 0.0011	< 0.001	<i>y</i> = 4.65 − 0.0162 <i>x</i>
4. Diffusion Gradient. Separate Experiment	29	−0.0200 ± 0.0001	< 0.001	<i>y</i> = 5.13 − 0.020 <i>x</i>

Notes.—Regressions calculated on *last two* figures of Specific Gravity determinations e.g. 1.072, and *first three* of the probit values, e.g. 4.3214.
Comparison of the residual variances of 1, 2, 3, namely 0.0342, 0.0249, and 0.0268 reveals no significant difference at the 5% level, therefore an overall regression has been calculated. This is − 0.0159 (*P* < 0.001) and gives an equation:
y = 4.60 − 0.0159 *x*.
Comparison between 1 and 4 reveals no significant difference.

Table II

Regressions (*b*) of probit per cent liver fat (dry and wet weight) and specific gravity, with (*n* = 5) and without (*n* = 4) 96 h value

Type of Measurement	<i>n</i>	<i>b</i> ± S. D.	<i>P</i>
Dry Weight <i>A</i>	5	- 0.0180 ± 0.0022	< 0.01 > 0.001
<i>A</i>	4	- 0.0171 ± 0.0023	< 0.05 > 0.01
Wet Weight <i>A</i>	5	- 0.0220 ± 0.0040	< 0.05 > 0.01
<i>A</i>	4	- 0.0200 ± 0.0040	< 0.05 > 0.01
Dry Weight <i>B</i>	5	- 0.0176 ± 0.0012	< 0.001
<i>B</i>	4	- 0.0172 ± 0.0013	< 0.01 > 0.001
Wet Weight <i>B</i>	5	- 0.0220 ± 0.0040	< 0.05 > 0.01
<i>B</i>	4	- 0.0200 ± 0.0040	< 0.05 > 0.01
Dry Weight <i>C</i>	5	- 0.0155 ± 0.0022	< 0.01 > 0.001
<i>C</i>	4	- 0.0146 ± 0.0013	< 0.01 > 0.001
Wet Weight <i>C</i>	5	- 0.0190 ± 0.0036	< 0.05 > 0.01
<i>C</i>	4	- 0.0170 ± 0.0001	< 0.001

Notes.—Regressions calculated as in Table I.

A = Specific Gravity by weighing, *B* = by Titration of whole liver, *C* = by Titration of Pieces.

Table III

Regressions (*b*) of probit per cent liver fat (dry weight) and specific gravity; log dose CCl₄ specific gravity and probit per cent liver fat

Type of Regression	<i>n</i>	<i>b</i> ± S. D.	<i>P</i>	Equation
1. Six groups of rats dosed with CCl ₄ and two control groups.				
Specific Gravity (<i>x</i>), Probit liver fat, (<i>y</i>); <i>x</i> and <i>y</i> are mean values of groups	8	- 0.0284 ± 0.0038	< 0.001	<i>y</i> = 6.86 - 0.028 <i>x</i>
2. Four groups of rats dosed 0.1, 0.2, 0.4, and 0.8 ml CCl ₄ /kg; five doses.				
Log (dose × 10) (<i>x</i>), Specific Gravity (<i>y</i>)	15	- 19.84 ± 5.00	< 0.01 > 0.001	<i>y</i> = 101.64 - 19.8 <i>x</i>
Log (dose × 10) (<i>x</i>), Probit per cent fat (<i>y</i>)	15	+ 0.50 ± 0.16	< 0.01 > 0.001	<i>y</i> = 3.11 + 0.50 <i>x</i>

paraffin were used. These had greater specific gravities than those normally recommended for the estimation of plasma proteins⁵.

In the second experiment the effects of a toxic agent (CCl₄) on the liver fat of rats were studied. Two groups of four rats per group were given a single dose of 1.0 ml/kg CCl₄ by intraperitoneal injection. Four groups were given 0.1, 0.2, 0.4, and 0.8 ml/kg on alternate days over a period of ten days. The animals were sacrificed, the liver specific gravities and fat determined as before. Good regressions were obtained for specific gravity and liver fat, log dose CCl₄, liver fat and specific gravity (Table III).

The experiments described suggest that there are considerable possibilities in the development of this technique for the assessment of liver fat in disease. How accurately a single determination can be made to forecast the fat content of the whole liver remains to be further investigated, but the degree of prediction is probably similar to that suggested by BILLING *et al.*¹. Further studies are

in progress to improve the accuracy of the technique and to investigate its applicability in the investigation of disease in animals.

I am indebted to Dr. J. D. BIGGERS for helpful discussions; to Dr. BARBARA BILLING for discussions and for drawing my attention to her studies while the present work was in progress, and to Dr. J. S. WILKINSON and Miss ANGELA HUNT for technical assistance.

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Résumé

Il existe un rapport étroit entre le poids spécifique et la teneur en matières grasses du foie. On peut le démontrer en utilisant des animaux empoisonnés avec du tétrachlorure de carbone.

⁵ E. A. KABAT and M. M. MEYER, *Experimental Immunochemistry* 1st Ed. (Charles Thomas, Springfield (Ill.) 1948).

DISPUTANDUM

Conformational Control of Bond Migration in the D-Homo Rearrangement of 17-Hydroxy-20-Keto Steroids

In a recent paper¹ with the above title, it is claimed that 'a convincing explanation for the differences in bond migration invoked by reagent as well as by steroid configuration at C-17 [in 17-hydroxy-20-keto steroids] has not yet appeared' and 'it is proposed that the factor controlling the bond migration in the rearrangement of these systems is a conformational one'.

As far as can be ascertained this is the first time that this 'unified rationale for the observed phenomena'¹, has been published in English. The same suggestion was, however, made some years ago in French² and experi-

mental support for it has since been obtained in the case of the Lewis acid catalysed rearrangement³.

IRÈNE ELPHIMOFF-FELKIN

*Centre National de la Recherche Scientifique, Paris,
July 7, 1959.*

In our recent article of the above title in *Experientia* we regret that through inadvertence on our part, we failed to cite an earlier exposition on this subject by I. ELPHIMOFF-FELKIN (*Bull. Soc. chim. 1956, 1845*) dealing with the application of this concept to the Lewis-acid catalyzed rearrangement.

N. L. WENDLER, D. TAUB,
and R. FIRESTONE

Merck & Co., Rahway, N. J., July 27, 1959.

¹ N. L. WENDLER, D. TAUB, and R. FIRESTONE, *Exper. 15*, 237 (1959).

² I. ELPHIMOFF-FELKIN, *Bull. Soc. chim. 1956, 1845*. See p. 1849: *Influence de la conformation de l'état de transition sur le résultat de la réaction.*

³ I. ELPHIMOFF-FELKIN and A. SKROBEK, *C. R. Acad. Sci., Paris 246, 2497* (1958); *Chem. Abstr. 52, 18524* (1958). Details in *Bull. Soc. chim. 1959, 742*.

Informations - Informationen - Informazioni - Notes

STUDIORUM PROGRESSUS

On Factors Involved in the Mechanism of 'Taste-Blindness'

By R. FISCHER and F. GRIFFIN*

Taste sensitivity to phenylthiourea, also called phenylthiocarbamide (PTC), and to other structurally related anti-thyroid compounds has been shown to follow a bimodal distribution^{1,2}. Recent data³ confirm the 7:3 ratio of tasters to non-tasters in a Caucasian population of adults and are in agreement with the genetic hypothesis that non-tasting is a single recessive character.

It has also been reported⁴ that it is the individual's own saliva that is necessary for tasting PTC and related compounds. The nature of this 'saliva factor', however, was not elucidated. FAWCETT and KIRKWOOD⁵ have presented evidence as to the presence of a soluble enzyme system, tyrosine iodine⁶, in the parotid and submaxil-

lary glands, and asked emphatically: 'What on earth is this enzyme system doing in the salivary glands?'⁸. However, a connection between tyrosine iodine and the 'saliva factor' was not suspected.

Recently, while confirming the existence of the disputed^{9,10} 'saliva factor' (to be published) we were fortunate in obtaining (through the courteous assistance of Dr. S. GARN, Yellow Springs, Ohio) a paper of N. TURNER *et al.*¹¹ reporting protein bound iodine (PBI) and total iodine values in the saliva of thirty children 8–14-years old from among the school population brought to the Forsyth Dental Infirmary for Children for dental examination and treatment. When analyzing the distribution of free iodine (which we obtained by deducting the PBI from the total iodine for each child) in the saliva of TURNER's subjects, we found that it resembled that of taste blindness to PTC and related compounds measured in young individuals¹². It is probable that the resemblance would be more striking if TURNER's subjects were a random sample rather than a 'dental' population and if the age group of both populations were exactly matched; the reason for this being that taste threshold for PTC is apparently distributed on a continuum at birth and becomes only gradually bimodal during the growth process.

Moreover, an examination of the data of WOLFE and TURNER¹³ shows that the amount of salivary peroxidase

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¹ H. HARRIS, *An Introduction to Human Biochemical Genetics*, Chap. VIII (Cambridge University Press, London 1955), p. 69.

² J. MOHR, *Ann. Eugen.*, London **16**, 282 (1951).

³ B. B. MERTON, *Acta Genet. statist. med.* **8**, 114 (1958).

⁴ J. COHEN and D. P. OGDON, *Science* **110**, 522 (1949).

⁵ D. M. FAWCETT and S. KIRKWOOD, *J. biol. Chem.* **209**, 249 (1954).

⁶ Which needs cupric ion and tyrosine to synthesize free moniodotyrosine; the system also requires hydrogen peroxide for the peroxidation of iodide⁷.

⁷ N. M. ALEXANDER, *J. biol. Chem.* **234**, 1530 (1959).

⁸ S. KIRKWOOD, *Chem. Canada* **7**, 23 (1955).

⁹ W. C. BOYD, *Psychol. Bull.* **48**, 71 (1951).

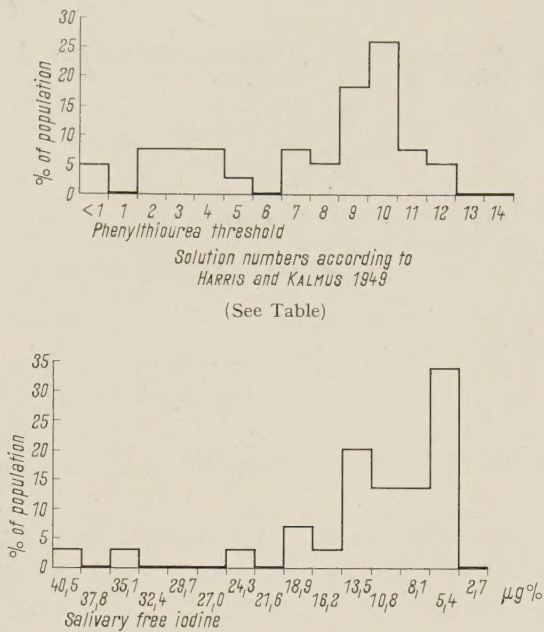
¹⁰ J. COHEN and D. P. OGDON, *Psychol. Bull.* **48**, 1 (1951).

¹¹ N. C. TURNER, P. H. DEMOGE, J. T. ANDERS, and G. E. CROWELL, *N. Y. State dent. J.* **22**, 134 (1956).

¹² H. HARRIS and H. KALMUS, *Ann. Eugen.*, London **15**, 24 (1949).

¹³ A. D. WOLFE and N. C. TURNER *J. dent. Res.* **36**, 843 (1957).

in 4–15-year old individuals is inversely related to the amount of salivary free iodine obtained from the data of TURNER¹¹.



Distribution of taste-blindness and free-salivary iodine in young individuals

Distribution of phenylthiourea threshold ('taste blindness') in 39 children 10–11 years of age from HARRIS and KALMUS, 1949 (top). Distribution of salivary free iodine in 30 children 8–14 years of age (from TURNER, 1956) (bottom)

Based on this (and other experimental) evidence¹⁴, we conclude that variations in taste sensitivity, of which taste blindness is an extreme form, are associated with genetic variations controlling the amount and composition of the soluble enzyme system, tyrosine iodinase ('saliva factor'). PTC and related compounds containing the HN–C=S grouping are apparently specific inhibitors of the soluble enzyme system. Their bitter taste can be modified by the very fact that the system will accept a number of substrates other than, but structurally related to, tyrosine¹⁵. The bitter taste of PTC and related compounds can also be modified by isosteric substitution which in turn will alter the specificity of these compounds

¹⁴ S. KIRKWOOD, *Enzymes Concerned with the Synthesis of Monoiodotyrosine*, in *Thyroid and Iodine Metabolism*, Report of the Twentieth Ross Pediatric Research Conference, Pittsburgh, Feb., 1956 (Issued by Ross Laboratories, Columbus, Ohio), p. 23.

¹⁵ On the differential reactivity of n-6-propylthiouracil with the 'saliva factor' of tasters and non-tasters determined with a 'Spectracord 4000-A' Perkin-Elmer recording spectrophotometer at $\lambda = 284 \mu$, the adsorption maximum of monoiodotyrosine. Experimental conditions: saliva diluted 1:5 and 'Tris' – buffered to pH 7.2 at 37°C; final concentrations of added n-6-propylthiouracil and monoiodotyrosine are $3.75 \times 10^{-4} M$ and $1.5 \times 10^{-4} M$, respectively.

Table

Phenylthiourea (PTC) solution numbers according to HARRIS and KALMUS, 1949	Molar concentration of PTC
1	8.5×10^{-3}
2	4.3×10^{-3}
3	2.1×10^{-3}
4	1.1×10^{-3}
5	5.3×10^{-4}
6	2.7×10^{-4}
7	1.3×10^{-4}
8	6.7×10^{-5}
9	3.3×10^{-5}
10	1.7×10^{-5}
11	8.3×10^{-6}
12	4.2×10^{-6}
13	2.1×10^{-6}
14	1.1×10^{-6}

as enzyme inhibitors. Such a concept may also account for the observation that from a representative sample of tasters and non-tasters ($N=31$) the four 'extreme non-tasters'¹⁶ profess to have significantly fewer food dislikes from a list of 120 foods than do tasters (to be published with S. GARN). However, there is no difference in taste threshold between these tasters and non-tasters towards the 'classical' taste qualities: sweet, salty, sour, and bitter.

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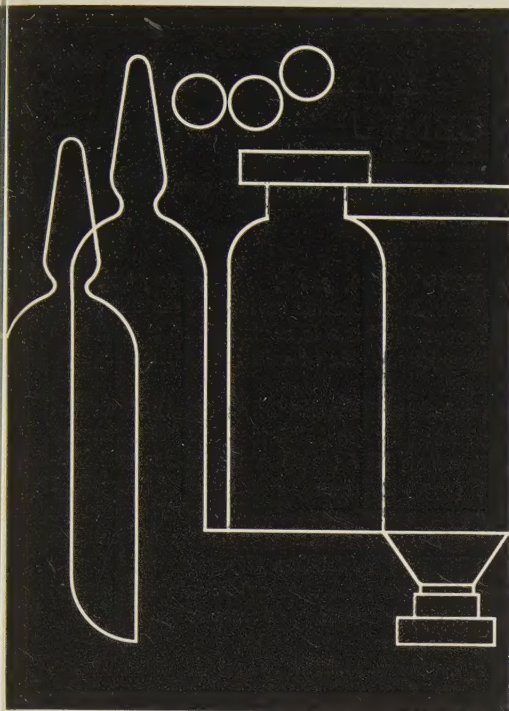
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Zusammenfassung

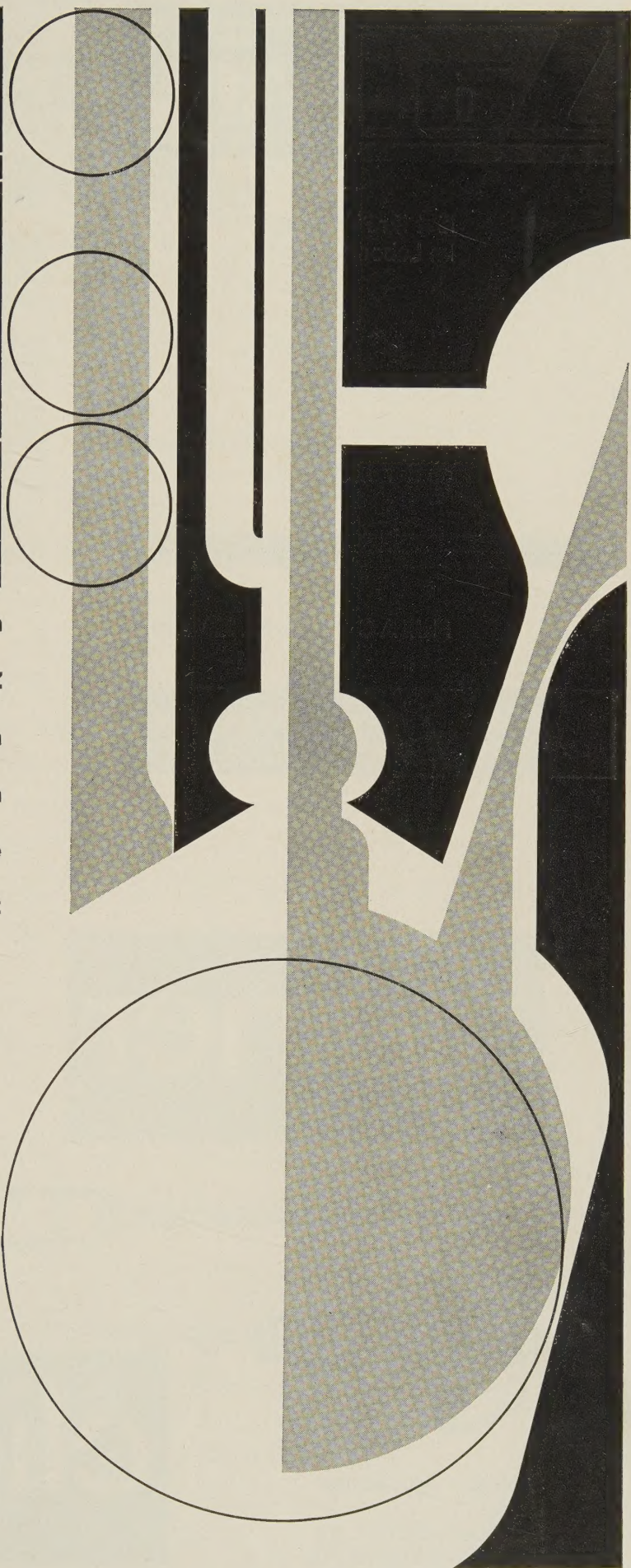
Der genetisch kontrollierte, hohe Geschmacksschwellenwert – Geschmacksblindheit – gegenüber Phenylthioharnstoff (PTC) und anderen bitteren, strukturell ähnlichen Anti-Schilddrüsensubstanzen, scheint durch die Qualität und Quantität des löslichen Speichel-Enzymsystems Tyrosiniodinase bedingt zu sein.

PTC-Geschmacksblinde sind «Alles-Esser», PTC-Schmecker hingegen weisen eine erhöhte kulinarische Selektivität auf. Diese Tatsache ist um so interessanter, als der Schwellenwert der beiden Gruppen gegenüber den klassischen Geschmacksqualitäten – süß, salzig, sauer und bitter – derselbe ist.

¹⁶ That is individuals barely able to differentiate a $6 \times 10^{-3} M$ 6-n-propylthiouracil solution (corresponding in molarity to No. 1 PTC solution of HARRIS and KALMUS) from placebos of distilled water when using the procedure of HARRIS and KALMUS¹.



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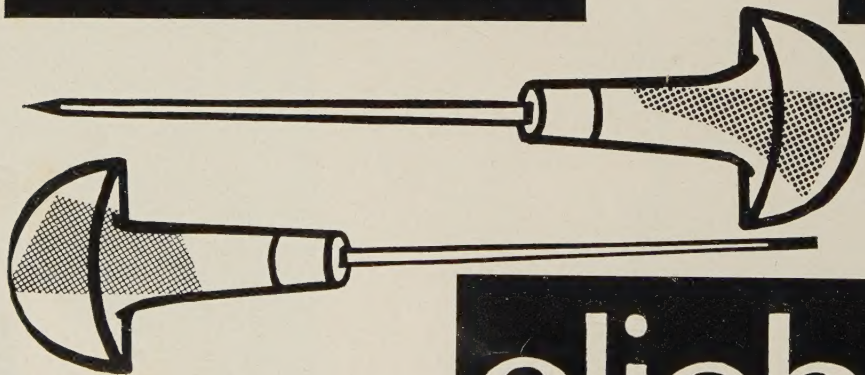
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